MOLECULAR STUDIES ON THE NEW ZEALAND TREE FERNS

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Abstract

Molecular phylogenetic studies have been performed on the New Zealand tree fern genera *Alsophila, Dicksonia* and *Sphaeropteris*, using DNA sequencing techniques and Amplified Fragment Length Polymorphism (AFLP). Three DNA sequence markers were used, two chloroplast: *rbcL*, *trnL-trnF* spacer, and one nuclear: *18S*. Using a combination of *rbcL* sequences obtained in this study and previously published sequences from GenBank an overall phylogeny for the tree fern clade is proposed. This phylogeny suggests that the currently recognised families may need revision. Phylogenetic analysis of molecular markers in *Dicksonia* suggests a three way genetic split within the genus, which corresponds, to three observed spore morphologies. AFLP studies on populations of *Dicksonia lanata*, which possesses two distinct growth forms, shows evidence of a weak genetic split, although probably not sufficient to warrant the separation of two species. Studies on the Cyatheaceae genera *Alsophila* and *Sphaeropteris* have confirmed observations on the evolution of the New Zealand species based on morphology, and have also suggested a heretofore unknown relationship between the South American fern genus *Hymenophyllopsis* and the Cyatheaceae.

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INTRODUCTION

1.1 The Tree Fern Clade

There are two commonly recognised families of tree ferns, the Dicksoniaceae Bower, and the Cyatheaceae Kaulfuss. These are mostly arborescent ferns forming large upright trunks, although a few species have prostrate stems (including New Zealand examples *Dicksonia lanata* Colenso and *Alsophila colensoi* J. D. Hooker), or rarer prostrate forms within normally upright members (e.g. *A. tricolor* (G. Forster) Colenso). Recent molecular phylogenies using *rbcL* DNA sequences have provided evidence for a monophyletic grouping of these two families together with five smaller families (Hasebe *et al.* 1995, Wolf *et al.* 1999). These additional families are the Metaxyaceae Pichi Sermolli, Lophosoriaceae Pichi Sermolli, Hymenophyllopsidaceae Pichi Sermolli, Loxsomataceae Presl and Plagiogyraceae Bower, none of which (with the exception of Lophosoriaceae) form true trunks. This grouping of families has been referred to as the 'tree fern clade' in recent molecular studies (e.g. Wolf *et al.* 1999).

Some other fern taxa are known to produce trunks. These include members of the Blechnaceae (e.g. *Blechnum* Linnaeus and *Sadleria* Kaulfuss), Thelypteridaceae, Dryopteridaceae and Osmundaceae. However, they are not phylogenetically closely related to the main taxa discussed in this work and will not be considered.

1.2 Dicksoniaceae

The Dicksoniaceae, as currently recognised, consist of 6 living genera, *Dicksonia* L'Heritier, *Cibotium* Kaulfuss, *Cystodium* W. J. Hooker, *Culcita* Presl, *Calochlaena* (Maxon) Turner & White and *Thyrsopteris* Kunze. There are approximately 40 species present in total within the family, more than half of which belong to the genus

Dicksonia. The Dicksoniaceae has in the past been united with the other tree ferns in a single family, united with the Pteridaceae in a superfamily, and broken into three separate families, Dicksoniaceae, Culcitaceae and Thyrsopteridaceae (*Lophosoria* Presl is sometimes included in the Dicksoniaceae, e.g. Pichi Sermolli 1977, or placed in it's own family, the Lophosoriaceae).

Ferns of the Dicksoniaceae generally have upright rhizomes although a few species are prostrate-creeping. The fronds are generally large, in the range of 1 - 3m. A defining characteristic of the family is the sporangia borne in sori on the underside margins of the leaf (as distinct from the Cyatheaceae, for example, whose sori are found away from the margins). The family has a largely Southern hemisphere distribution. *Dicksonia*, *Cibotium*, *Calochlaena* and *Culcita* are found in the neo- and paleotropics, *Thyrsopteris* is found on the Juan Fernandez Islands and *Cystodium* in Malesia. *Culcita* is also found in Spain, Portugal and Madeira, as well as South America. Fusion of indusia, the protective covering of the spore-bearing sori, is regarded as a derived character, while separate indusia, as found in *Calochlaena dubia* (R. Brown) Turner & White, are regarded as primitive (Tryon & Tryon 1982).

Fern phylogeny based on analysis of *rbcL* sequences places the Dicksoniaceae in a monophyletic group alongside the Cyatheaceae and related families including the Metaxyaceae, Lophosoriaceae and Hymenophyllopsidaceae (Hasebe *et al.* 1995, Wolf *et al.* 1999). The diversification of this so called 'tree fern clade' appears to have occurred early in the evolution of leptosporangiate ferns. It is interesting that *rbcL* data tends to place *Dicksonia* closer to *Culcita* and *Calochlaena,* and somewhat more distant to *Cibotium* (e.g. Hasebe *et al.* 1995), whereas analysis of morphological data has led to the conclusion that *Dicksonia* is closely related to *Cibotium* and is more distant to the other groups (e.g. Stevenson & Loconte 1996). *Culcita* and *Calochlaena* have sometimes been placed in their own group (the Culcitaceae Pichi Sermolli). This illustrates the contradictions between molecular and morphological data used to resolve evolutionary relationships. Both forms of data are subject to bias and only by

careful analysis can this be understood. Some morphological characters are more variable than others, e.g. chromosome numbers and spore morphology tend to be more conserved than vegetative characters (Smith 1995). Similarly in DNA some bases may be more prone to variability e.g. third codon positions in coding sequences are less conserved than first and second codon positions. Agreement between the two forms of data will hopefully give a more reliable result. The position of *Cibotium* closer to the Cyatheaceae than to *Dicksonia*, *Culcita* and *Calochlaena* suggests the Dicksoniaceae may be a paraphyletic group. This agrees with chromosome counts for these taxa. *Dicksonia* (n = 65), *Culcita* and *Calochlaena* (n = 66) are similar, as are *Cibotium* (n = 68) and the Cyatheaceae (uniformly n = 69), and perhaps indicates the *rbcL* analysis is reliable. *Thyrsopteris* and *Cystodium* in the Dicksoniaceae have not been assessed using *rbcL*. However, these are considered to be more distant to the other genera in the family, supported by cytology and spore morphology (e.g. chromosome count for *Thyrsopteris*: n = 76 - 78, and an unconfirmed count for *Cystodium* of n = 56, J. Knouse pers. comm.). *Cystodium* has sphaeroidal, granulate spores which are similar to those of *Metaxya* (Tryon & Lugardon 1991).

1.2.1 *Dicksonia*

Dicksonia contains about 25 living species. The genus was first described by L'Heritier in 1788 in *Sertum anglicum* with *Dicksonia arborescens*, the type species for the genus. The genus derives its name from Scottish botanist James Dickson. *Dicksonia* comprises a group of large, terrestrial ferns with dictyostelic stems usually erect up to 10m. These stems are often massive, bearing long, dense hairs and many fibrous roots. Some species have a creeping rhizome in contrast to an upright stem (e.g. *Dicksonia lanata* in New Zealand). The fronds of *Dicksonia* species are usually 1 - 3.5 m long and borne spirally in a spreading crown. The lamina are 2-pinnate-pinnatifid to 4-pinnate. Sori are found on the underside of the pinnae at the margins, a trait characteristic of the family, and have indusia which envelop the sporangia. The chromosome count for *Dicksonia* is n = 65 (Tryon & Tryon 1982).

The genus can be divided into three groups based on spore morphology (Large & Braggins 1991, Q. Cronk pers. comm.). All spores are tetrahedral-globose and trilete, but differ with respect to their surface texture. The spores of the first group have a smooth surface relative to the others, although it is somewhat granulate. The group is represented in New Zealand by *D. fibrosa*. The second group has spores which have a tuberculate surface texture. The representative New Zealand species are *D. lanata* and *D. squarrosa* (G. Forster) Swartz. The final group consists of only a single species, *D. arborescens*. The spores of this species are intermediate between those of the other two groups. One side of the spore is smooth and the other is tuberculate.

1.2.2 Habitat and Distribution

Dicksonia species generally prefer wet mountain forests, especially in the tropics. In more temperate regions they are also found in more open areas, such as thicket-scrub and exposed wet slopes. They occur at altitudes up to about 3500 m (in South America, Tryon & Tryon 1982).

Members of the genus follow a distribution pattern related to their spore morphology. The species with a smooth spore structure are distributed throughout South America, the Juan Fernandez islands, Australia and New Zealand. Species of the tuberculate spore group are common throughout Papuasia, Australia and New Zealand. New Zealand is situated at the intersection of these two spore groups (as is the Eastern Coast of Australia). There are significantly more species in the tuberculate spore group than the smooth spore group (see Table 1.1). *Dicksonia arborescens*, the lone member of the intermediate spore group, resides solely on St. Helena island in the Atlantic.

Dicksonia species			
Name	**Authority**	**Distribution**	**Spores**
D. antarctica	Labillardiere	Australia	granulate
D. fibrosa	Colenso	New Zealand	granulate
D. sellowiana	W. J. Hooker	Mexico to S. America	granulate
D. steubelii	Hieronymus	Peru	granulate
D. arborescens	L'Heritier	St. Helena	intermediate
D. archboldii	Copeland	New Guinea	tuberculate
D. baudouinii	Fournier	New Caledonia	tuberculate
D. berteriana	(Colla) W. J. Hooker	Juan Fernandez	tuberculate
D. blumei	(Kunze) Moore	Java to New Guinea	tuberculate
D. brackenridgii	Mettenius	Vanuatu to Fiji	tuberculate
D. grandis	Rosenstock	New Guinea	tuberculate
D. herbertii	W. Hill	Australia	tuberculate
D. hieronymi	Brause	New Guinea	tuberculate
D. lanata	Colenso	New Zealand	tuberculate
D. lanigera	Holttum	New Guinea	tuberculate
D. mollis	Holttum	Java to New Guinea	tuberculate
D. sciurus	C. Christensen	New Guinea	tuberculate
D. squarrosa	(G. Forster) Swartz	New Zealand	tuberculate
D. youngiae	Moore ex Baker	Australia	tuberculate
D. externa	Skottsberg	Juan Fernandez	tuberculate
D. thyrsopteroides	Mettenius	New Caledonia	tuberculate

Table 1.1 *Dicksonia* Species List: including 21 known species, with distribution and general spore surface morphology (all spores are trilete and terahedral-globose). Granulate spores possess a smooth surface with fine granulate perine, tuberculate spores have a knobbled surface texture and intermediate spores possess both granulate and tuberculate faces. Note: several species from New Guinea are awaiting description (R. Johns pers. comm.).

1.2.3 *Dicksonia* in New Zealand

In New Zealand there are three species: *Dicksonia lanata*, *D. squarrosa* and *D. fibrosa*, all considered endemic.

Dicksonia fibrosa was first described by Colenso in 1845. It is referred to as wheki-ponga or kuripaka as its Maori name. It grows to 6 m in height, typically with a thick trunk. It has numerous fronds forming a dense crown at the top of the trunk. The fronds are typically 1.5 - 3 m in length and when dead form a thick, persistent skirt obscuring the trunk. The stipes of the fronds are pale brown in colour which distinguishes it from *D. squarrosa*. The spores of this species are smooth, placing it in the same spore group as the South American *D. sellowiana* and *D. antarctica* of

Australia. *D. fibrosa* is found in the North, South, Stewart and Chatham Islands, but is common only in the central districts of the North Island. It is a subcanopy species in lowland to montane forest or semi-open country in the North Island, while in the South Island is mostly found in coastal or lowland regions.

Dicksonia squarrosa was first described by G. Forster in 1786 as *Trichomanes squarrosum*. It was later assigned to the genus *Dicksonia* as *D. squarrosa* by Swartz in 1802. It is commonly known as the rough tree fern or wheki and reaches about 7 m in height, with a thin trunk (compared to *D. fibrosa*). It sometimes forms a two-headed plant (occasionally more) if the crown or upper stem is damaged. Buds on the main trunk are able to grow, as in some of its Australian counterparts (*D. herbertii*, *D. youngiae* and occasionally *D. antarctica*). It often forms groves due to the presence of underground stolons. The fronds of this fern are again about 1.5 - 3 m in length, and form an untidy crown. The dead fronds form a scruffy skirt which, combined with the live fronds, gives the fern a characteristic "scrappy" appearance. The stipes of the fronds are black, which provides a rigid distinction from its similar New Zealand counterpart, *D. fibrosa*. The spores of *D. squarrosa* are tuberculate, placing it in the same spore group as the Papuasian species. This fern is common in both the North and South Islands. It is also found on Stewart Island, the Chatham Islands and the Three Kings Islands. It is a subcanopy species in coastal to montane forest, and often remains after bush has been destroyed.

Dicksonia lanata was described by Colenso (1845). It is a much smaller tree fern than either of the other New Zealand species. It is present in two growth forms within New Zealand. One is erect, with a slender stem up to 2 m in height, the other is a prostrate and stoloniferous. The fronds are also proportionately smaller, being only 0.75 - 2 m in length. The stipes are pale brown, and long, being anywhere from half to as long as the laminae. The spores of this plant are tuberculate as in *D. squarrosa*. *D. lanata* is not found offshore, unlike the other species, it is confined to the mainland.

The two different growth forms of *D. lanata* are both physiologically and geographically distinct. The upright form is found only in the upper North Island, from North Cape to the Coromandel, in lowland Kauri (*Agathis australis* Salisbury) forests. The creeping form is more widespread and is found throughout the central and more rarely, the lower North Island and the north-western South Island, from the Malborough Sounds to Franz Josef. This form is found in montane forest or open scrub in the North Island, and lowland and coastal forests in the South Island. Indeed, this variation was recognised by Colenso when he first described the species. He recorded the Northern form as *D. lanata* var. *hispida* also in 1845, shortly after publishing the species.

1.3 Cyatheaceae

This is the largest tree fern family, with approximately 600 species world-wide, and a Southern temperate distribution. The name is derived from the Greek word *cyathos,* meaning 'cup', in reference to the shape of the indusia in some species. The Cyatheaceae are sometimes referred to as the scaly tree ferns, differentiated from the Dicksoniaceae which are known as the hairy tree ferns. Considering the diversity among the Cyatheaceae it is perhaps remarkable that all recorded chromosome counts are of n=69.

The classification of the Cyatheaceae into separate genera has been problematic. It has been broken into genera in several different ways, based on morphological characters (see figure 1, Conant *et al.* 1994). Holttum and Edwards (1983) united the Cyatheaceae into a single genus *Cyathea* J. Smith, with two subgenera, *Cyathea* subg. *Cyathea* and *Cyathea* subg. *Sphaeropteris.* Tryon and Tryon (1982) recognised six different genera, *Cnemidaria* Presl, *Cyathea, Alsophila* R. Brown, *Sphaeropteris* Bernhardi, *Nephelea* Tryon, and *Trichipteris* Presl. Most recently, Lellinger (1987) proposed four genera, *Cnemidaria, Cyathea, Alsophila,* and *Sphaeropteris.* The generic

boundaries circumscribed by the different authors also differ. Tryon and Tryon's *Sphaeropteris* for example, is not equivalent to Lellinger's *Sphaeropteris*, as Lellinger shifted Tryon and Tryon's *Sphaeropteris* subgenus *Schlephropteris* species into *Cyathea*.

In an attempt to address this problem a group led by David Conant has utilised chloroplast restriction site data as an additional source of data to morphological characters (Conant *et al.* 1994, 1996). Their work to date, on a large number of species, seems to agree most closely with the system proposed by Lellinger, with one exception, *Cyathea* and *Cnemidaria* appear to form a single monophyletic group, suggesting that they be merged into a single genus. This work is still in progress and no new taxonomic scheme is yet available. This thesis follows Lellinger's classification scheme for the Cyatheaceae.

Aside from Conant's chloroplast restriction site studies, little molecular data has been obtained from the Cyatheaceae. Data from *rcbL* sequencing in the Cyatheaceae has involved few species, and it appears that all currently published sequences are of the genus *Sphaeropteris*. Analyses of these data have suggested a sister relationship with the Hymenophyllopsidaceae, although more sequences from the Cyatheaceae would be needed to clarify this relationship. A more distant alliance to the genus *Cibotium* of the Dicksoniaceae has also been suggested (e.g. Wolf *et al.* 1999), indicating the Dicksoniaceae may be paraphyletic.

The Cyatheaceae are also an ancient family, with a fossil record to the Late Jurassic - Early Cretaceous, e.g. *Oguracaulis* Tidwell (Tidwell *et al.* 1989). However, modern genera such as *Cyathea* and *Cnemidaria* do not show up in the fossil record until relatively late in the Tertiary (Collinson 1996).

1.3.1 Spore Morphology

The extant Cyatheaceae have quite a wide variety of spore architectures but have a consistently trilete apeture and tetrahedral-globose shape. Spores of *Alsophila* generally possess raised ridges, and some species such as *A. tricolor* may have a granulate deposit forming rodlets over the surface. A few species may possess pitted spores. *Sphaeropteris* spores are often plain with coarse, echinate elements on low ridges, or sometimes granulate or verrucate without rodlets. Rarely they are verrucate-foraminate. *Cyathea* possesses spores which have either a plain or verrucate surface with rodlets. They are sometimes pitted. A few species are irregularly verrucate with no rodlets. *Cnemidaria* spores are very distinctive, usually having three large pits located in the equatorial area between aperture arms, and a scattering of smaller pits (Tryon & Lugardon 1990).

Spores of the New Zealand Cyatheaceae have been described as falling into three categories (Large & Braggins 1991). Spores of *Alsophila colensoi*, *A. cunninghamii*, and *A. kermadecensis* have a granulate surface with raised ridges, typical of many *Alsophila* species. *Alsophila smithii* has a smooth to scabrate surface architecture unique among the New Zealand species. Finally, *Alsophila tricolor*, *A. milnei* and *Sphaeropteris medullaris* have spores with a spinulate surface architecture.

Conant *et al.* (1996) found an overall congruence of spore architecture with evolutionary lineage's proposed from chloroplast (cp) DNA data. For example, the verrucate architecture was confined to their proposed *Cyathea divergens* Kunze clade, the plain spore type was almost exclusive to the *Trichipteris gibbosa* (Klotzsch) Barrington and *T. armata* (Swartz) Tryon clades, and the ridged spore type is restricted to *Alsophila*. These and other morphological characters mapped onto their cpDNA tree supported the proposed groupings.

1.3.2 Habitat and Distribution

Cyatheaceae have a wide distribution in the Southern Hemisphere and montane tropics. Species are found throughout Africa, South East Asia, South America and the Pacific, including Australia and New Zealand. The genera vary in their distributions, with *Alsophila* and *Sphaeropteris* being found predominantly in the Old World, with small groups of species found in the New World tropics. *Cyathea* is found in large numbers in both the New World and the Old World, and *Cnemidaria* is found solely in the New World tropics.

The Cyatheaceae prefer wet, mountainous regions, and it is in these areas that the most species diversity is observed (Tryon and Gastony 1975). These include Madagascar, New Guinea, Central America and Chile. They are found at altitudes from sea level up to 4200 m in the Andes. The majority of species reside at 1000 – 1500 m.

1.3.3 Cyatheaceae in New Zealand

There are seven species within the Cyatheaceae in New Zealand. These are most commonly recognised as being in a single genus, *Cyathea*. However, in following the most recent classification scheme (Lellinger, 1987, closest to the results of Conant *et al.* 1996), this thesis recognises two genera, *Alsophila* and *Sphaeropteris*.

Sphaeropteris

Sphaeropteris medullaris (G. Forster) Bernhardi. is the lone member of the genus *Sphaeropteris* present in New Zealand and is the type for the genus. It was first described as *Polypodium medullare* by G. Forster in 1786. It was subsequently placed in *Cyathea* as *C. medullaris* by Swartz in 1801. In the same year it was also assigned to the genus *Sphaeropteris* by Bernhardi. *S. medullaris* grows up to 20 m tall with

fronds to 5 m. Stipes are thick and black, and persistent only in immature plants. Marginal spines on the stipe and lamina scales distinguish it from other members of the family in New Zealand. It has a wide distribution throughout New Zealand and in the Pacific Islands. In New Zealand it is found in lowland forest throughout the North Island and along the coast in the South Island. In the chloroplast DNA studies of Conant *et al.* (1994, 1996) *S. medullaris* appears to be a basal member of the *Sphaeropteris* clade.

Alsophila

There are 6 species in the genus *Alsophila* found in New Zealand. *A. colensoi, A. cunninghamii, A. kermadecensis, A. milnei, A. smithii* and *A. tricolor.* These species were not represented in the chloroplast DNA studies of Conant *et al.* (1994, 1996), and as such, have not been previously studied using molecular methods.

Alsophila colensoi was first described in 1854 by J. D. Hooker. It was later moved to *Cyathea* by Domin in 1929. *Alsophila colensoi* has either a prostrate stem or rarely a short, erect trunk up to 1 m tall, and is often mistaken for a young *A. smithii.* This characteristic, and the absence of indusia, distinguish it from other New Zealand species. It has slender, pale brown stipes and fronds up to 1.5 m in length. It is found in montane forests throughout the North, South and Stewart Islands.

Alsophila cunninghamii was first described by J. D. Hooker in 1854 as *Cyathea cunninghamii.* It was later assigned to *Alsophila* by Tryon (1970). It can grow up to 20 m tall and has fronds up to 3 m in length. The stipes are dark brown and slender. It is very similar to *A. kermadecensis* and is distinguished from it by the presence of stellate hairs on the underside of the lamina. *A. cunninghamii* is found in both Australia and New Zealand.

Alsophila kermadecensis was first described by W. R. B. Oliver in 1910 as *Cyathea kermadecensis*. It was subsequently assigned to *Alsophila* by Tryon (1970) as *A. kermadecensis*. This fern is very similar to *A. cunninghamii,* growing up to 20 m tall with fronds up to 4 m. Stipes are slender and covered in hairs and scales. Distinguished from *A. cunninghamii* by the absence of stellate hairs on the underside of the lamina, and the lack of dead stipe bases on the trunk. *A. kermadecensis* is endemic to the Kermadec Islands.

Alsophila milnei was first described as *Cyathea milnei* in 1864 by J. D. Hooker. Tryon (1970) later assigned it to *Alsophila*. It grows to 8 m tall and has large fronds up to 4 m. The stipes of this fern are pale brown and rough, covered in scales. This plant is though to be closely related to *A. tricolor* but distinguished by the absence of the white bloom on the underside of the fronds. *A. milnei* is endemic to the Kermadec Islands.

Alsophila smithii was again first described by J. D. Hooker, as *Cyathea smithii* in 1854. It was later reassigned as *A. smithii* by Tryon (1970). This fern grows up to 8 m tall and has fronds up to 2.5 m in length. The stipes of this plant are slender and pale to dark brown. *A. smithii* is easily recognised by its persistent skirt of dead stipes. The only other New Zealand tree fern also forming a persistent skirt is *Dicksonia fibrosa* (of full fronds). *Alsophila smithii* is found in the North, South, Stewart, Chatham and Auckland Islands. It has the Southern-most distribution of any tree fern in the world.

Alsophila tricolor was first described as *Polypodium dealbatum* by G. Forster in 1786. It was moved to *Cyathea* as *C. dealbata* by Swartz in 1801, which is still commonly accepted. It was first given the name *A. tricolor* by Colenso, so when returned to *Alsophila* by Tryon (1970) it assumed this name, as this was the first published name for it in *Alsophila*. *A. tricolor* has a trunk up to 10 m tall, with fronds up to 4 m. Stipes are silver when young, becoming pale brown later. It is found throughout the

North and South Islands, also Three Kings and Chathams, in dry forest and open scrub. This plant is commonly referred to as silver fern, and is one of New Zealand's national emblems.

Name	Trunk	Frond	Stipes	Indusium	Frond Hair	Frond Scales	Spores
S. medullaris	20m	5m	black	complete	none	spiny margins	spinulate
A. colensoi	1m	1.5m	light brown	none	red stellate	stellate spines	ridged
A. cunninghamii	20m	3m	black/brown	hood	red stellate	stellate spines	ridged
A. kermadecensis	20m	4m	brown	hood	none	stellate spines	ridged
A. milnei	8m	4m	light brown	deep cup	curly hair	none	spinulate
A. smithii	8m	2.5m	light brown	saucer	red stellate	spineless	smooth
A. tricolor	10m	4m	light brown	deep cup	curly hair	none	spinulate

Table 1.2 Comparative Morphology of NZ Cyatheaceae: *S.* = *Sphaeropteris*, *A.* = *Alsophila*. Trunk = maximum trunk height, Frond = maximum frond length, Stipes = colour of stipes, Indusium = shape of indusium, Frond Hair/Scales = type of hair and scales found on fronds, Spores = spore surface architecture (compiled from Brownsey & Smith-Dodsworth 1989, Large & Braggins 1991).

1.4 Related Families

The Lophosoriaceae consists of a single genus, with arguably only a single species, *Lophosoria quadripinnata* (Gemlin) C. Christensen. This fern forms upright trunks to 5 m with fronds 0.3 - 5 m in length, and sporangia in sori on fertile veins. It is found in South America and the Juan Fernandez Islands. Data from *rbcL* places the genus *Lophosoria* as sister to *Dicksonia* (Wolf *et al.* 1999). This agrees well with the chromosome count for both genera of n = 65. Fossil *Lophosoria* (foliage and spores of the fossil species *L. cupulatus* D. J. Cantrill) has been found in Lower Cretaceous strata in Antarctica (Cantrill 2000), and Miocene deposits in Costa Rica (Graham 1987) as well as throughout Australia (Hill & Jordan 1998), suggesting it was once widespread.

The Hymenophyllopsidaceae is again monogeneric. *Hymenophyllopsis* Goebel, contains about 8 species. These are small ferns with short creeping or upright stems and fronds generally ranging from 10 - 40 cm, very similar in appearance to members

of the genus *Hymenophyllum* Smith. However similarities (i.e. thin leaves lacking stomates) are probably adaptive and not indicative of relatedness (Tryon & Tryon 1982). Sporangia have an oblique annulus which has been compared to that of *Alsophila* (Christensen 1938) and are borne in marginal sori. *Hymenophyllopsis* occurs only in the Guayana Highlands of Venezuela, the most narrow geographic range of any fern family (Wolf *et al.* 1999). Affinities based on morphological characters have varied (e.g. Stevenson and Loconte 1996, Lellinger 1995), however, putative relationships based on *rbcL* data places this genus as sister to the Cyatheaceae (Wolf *et al.* 1999), which is in agreement with spore and scale morphology.

Metaxyaceae is another family with a single genus: *Metaxya* Presl, and a single species: *M. rostrata* (Kunth) Presl. This species is prostrate to nearly erect, with fronds 1 - 2 m in length. Chromosome counts of n = 94 - 96 are reported (Tryon & Tryon 1982). This fern is found in Central and Southern America. It has been considered related to the tree ferns, as part of the "proto-Cyatheaceae" with *Lophosoria*, within the Cyatheaceae, or in a large family including both the Cyatheaceae and Dicksoniaceae. It is most likely allied, but not closely related to these families (Tryon & Tryon 1982). *Metaxya rostrata* possesses unusual sphaeroidal, granulate spores.

The family Plagiogyriaceae has a single genus, *Plagiogyria* (Kunze) Mettenius, of about 15 species, although as many as 30 have been described. The distribution of this family is centered in China, with one species in America. These ferns possess a short stem, decumbent to erect, and fronds up to 2 m. Sporangia are borne in sori on the veins of the pinnae. Chromosome counts vary, although n = 66 and n = 132 seem the most reliable (Tryon & Tryon 1982), indicating some polyploidy. This family has been considered an isolated one, with similarities to the Osmundaceae, or more advanced ferns such as *Blechnum* Linnaeus, although this is supported by few characters.

The Loxsomataceae is again a small family, containing three species in two genera. One monotypic genus *Loxsoma* Cunningham is endemic to the North Island of New Zealand. The species is *L. cunninghamii* Cunningham, for which a chromosome count of n = 50 has been recorded (Brownsey 1975). The genus name was originally spelt *Loxoma*, but was corrected to *Loxsoma* by Endlicher and Allan (see Brownsey *et al.* 1985). The second genus, *Loxsomopsis* Christ (n = 46) contains two species: *L. costaricensis* Christ and *L. notabilis* Slosson with an American distribution, from Costa Rica to the Andes of Colombia, Peru and Bolivia. Ferns of both genera possess a long, creeping stem and fronds ranging from 0.5 - 5 m in length. Sporangia are borne in marginal sori. The family has been considered an isolated one but with affinities to the Dennstaedtiaceae. Early work also noted similarities to the Hymenophyllaceae and Cyatheaceae (Tryon & Tryon 1982).

1.4.1 Morphological vs. Molecular Studies

Classifications based on morphological characters for these families have varied. For example, Hymenophyllopsidaceae has not been considered related to the tree fern families Cyatheaceae and Dicksoniaceae, although similarities with the Cyatheaceae have been noted. Stevenson and Loconte (1996) produced a phylogenetic tree based on morphological characters that included all but Hymenophyllopsidaceae in the 'tree fern clade'. Hymenophyllopsidaceae was placed as a basal branch of the Hymenophyllacae, although the authors noted a lack of data from this taxon. The Metaxyaceae and Lophosoriaceae have long been considered related to the tree ferns, but are still considered as separate families. The Loxsomataceae and Plagiogyriaceae have both been considered as isolated families, with affinities to the Dennstaedtiaceae and Osmundaceae, respectively (Tryon & Tryon 1982).

In contrast, analyses of *rbcL* sequence data from these families suggests they form a single monophyletic evolutionary group, but has been unclear on the relationships between them. Phylogenies have varied by study and by tree-building methods used

(e.g. Hasebe *et al.* 1995). This may be in part due to an increased frequency of mutation at synonymous sites (estimated to be approximately 20 times greater than non-synonymous sites (Wolf 1996). However, some relationships seem fairly clear. The Loxsomataceae and Plagiogyriaceae consistently appear as sister taxa, although they are not closely related. Wolf *et al.* (1999) report that the genera *Lophosoria* and *Dicksonia* are closely related, as are *Sphaeropteris* (reported as *Cyathea*) and *Hymenophyllopsis*.

1.5 Tree Fern Fossil Record

Extant tree ferns have a Southern Hemisphere and montane tropical distribution, although the fossil record shows that they were once more widespread throughout the Northern Hemisphere. These families have ancient fossil records extending back as far as 200 million years (see Table 1.3). Fern spores make especially good fossils, as they are made of sporopollenin, the most resistant biological polymer known. Spore morphology is considered to provide a highly stable phylogenetic character, changing little in millions of years (Large & Braggins 1991, Harris 1955). Spores are the most common type of fossil found. However, fossilised fronds are most useful in identifying ancient species, but these are relatively rare. Fossilised trunks are also rare, and useful for identifying a fossil find as a tree fern. Fossil dates are increasingly being used in molecular studies to calibrate phylogenetic trees in order to estimate times of divergence between taxa.

1.5.1 Dicksoniaceae

The Dicksoniaceae have a fossil record into the Triassic (248 - 213 mya), and may have origins in the Paleozoic (590 - 248 mya, Collinson 1996). The family has probably undergone retreat into the Southern Hemisphere during the Tertiary (65 - 2 mya). Fossils are recorded from several genera, some of which are known only as

fossils. These include extant *Dicksonia* and *Cibotium*, and extinct *Coniopteris* and *Erboracia*. This evidence suggests an early diversification within Dicksoniaceae with later extinctions. Fossils are frequently found in regions far distant from locations of the current distribution of the Dicksoniaceae, such as Siberia and Yorkshire (Van Konijnenberg-Van Cittert 1989). This observation may suggest that these plants were once a dominant genus in many regions across the world and that the current distribution is relic.

The oldest known fossil tree fern genus is *Coniopteris*, recorded from the Triassic of Iran. It is best compared with the extant genus *Thyrsopteris*. Other fossil genera, *Onychiopsis*, known from the Cretaceous (144 - 65 mya) of Denmark, and *Erboracia*, recorded from the Jurassic of Yorkshire, are also compared to modern *Thyrsopteris*. Far more *Thyrsopteris*-like fossils are observed than *Dicksonia* in the fossil record. The modern genera *Thyrsopteris* and *Dicksonia* may have diverged as far back as the Triassic. Fossils of *Cibotium* have been found in late Jurassic - early Cretaceous strata in Tasmania, and *Nishidacaulis*, an American fossil genus compared to extant *Cibotium*, dates to the early Cretaceous. It has been suggested that speciation within *Cibotium* took place in the mid-Eocene (54.9 - 38 mya, Tidwell & Nishida 1993). *Dicksonia* has the longest fossil record of the extant genera, with confirmed fossils from the Jurassic (213 - 144 mya) in Siberia and Yorkshire. Fossilised *Conantiopteris* from the late Cretaceous appears to be similar to extant *Dicksonia* and *Lophosoria*. This long fossil record suggests that genera currently placed in the Dicksoniaceae diverged a very long time ago.

1.5.2 Dicksonia

Within *Dicksonia* fossils, two lineages are observed, corresponding to two of the modern spore architectures. Granulate spores are known from the oldest *Dicksonia* fossils, dating back to the Jurassic similar to modern species of Australia, New Zealand and South America (e.g. *D. antarctica, D. fibrosa, D. sellowiana*). Tuberculate

(knobbled) spores turn up much later in the fossil record, not until well into the Tertiary. Many spores have come from regions far outside the current distribution of the genus, e.g. Siberia (Van Konijnenberg-Van Cittert 1989).

Spores have also been discovered within the current distribution range of *Dicksonia*. One such example is *Dicksonia dissecta* Jordan, Macphail and Hill, a fossil species found in early Oligocene (38 - 24.6) sediments at Little Rapid River in western Tasmania (Jordan *et al.* 1996). In this case a fertile frond fragment was discovered containing both sporangia and spores. The spores of this species were tuberculate placing it in the same spore group as the Papuasian species, and some of the Australian and New Zealand species (e.g. *D. baudouinii*, *D. herbertii*, *D. lanata*). Within New Zealand fossil spores have been discovered dating back to the Late Oligocene-Early Miocene period. It has been postulated that these were produced by *Dicksonia dissecta* or a related species (Jordan *et al.* 1996). As Jordan *et al.* suggest, it is possible that the divergence of these spore types occurred late in the Paleocene (65 - 54.9 mya).

1.5.3 Cyatheaceae

Cyatheaceae fossils are known from the earliest Cretaceous (about 144 mya). Fossil genera such as *Oguracaulis*, *Cyathocaulis* and *Cyathodendron* are known from this period. Two series have been identified among the Cyatheoid fossils, one consisting of *Oguracaulis* , *Cyathocaulis* and other scaly Cyatheoids, and a second of *Cibotiocaulis*, *Paracyathocaulis* and modern genera such as *Cyathea* and *Cnemidaria* (Collinson 1996). Modern Cyatheaceae genera only show up in the fossil record relatively late in the Tertiary (e.g. *Cnemidaria* from Eocene deposits in the USA). Fossils of the families related to the Dicksoniaceae and Cyatheaceae are not so well known. However, *Lophosoria* is an exception, with fossils dating from the lower Cretaceous from Antarctica, as well as in Miocene deposits in Costa Rica (24.6 - 5.1 mya) and throughout Australia.

Taxon	Authority	Locality	Triassic 248-213 Early	Triassic 248-213 Late	Jurassic 213-144 Early	Jurassic 213-144 Late	Cretaceous 144-65 Early	Cretaceous 144-65 Late	Tertiary 65-2 Early	Tertiary 65-2 Late
Cyatheaceae	Kaulfuss									
Extinct										
Cyatheocaulis	Ogura	Korea								
Oguracaulis	Tidwell *et al.*	Tasmania								
Alsophilocaulis	Menendez	Argentina								
Cibotiocaulis	Ogura	Korea								
Extant										
Cnemidaria	Presl	Australia								
Cyathea	J. Smith	USA								
Dicksoniaceae	Bower									
Extinct										
Coniopteris	Brongniart	Iran								
Nishidicaulis	Tidwell & Nishida	Australia								
Onychiopsis	see Tidwell & Ash	Denmark								
Conantiopteris	Lantz *et al.*	USA								
Erboracia	H. H. Thomas	England								
Extant										
Calochlaena	Turner & White	Australia								
Cibotium	Kaulfuss	Australia, USA				?	?			
Dicksonia	L'Heritier	Europe				1				2
Lophosoriaceae	Pichi Sermolli									
Extinct										
Lophosoriorhachis	Nishida	Japan								
Extant										
Lophosoria	Presl	Antarctica								

Table 1.3 Tree Fern Fossil Record: Selected fossil tree fern genera. ? = Likely a *Cibotium* ancestor rather than a true *Cibotium*. 1 = *Dicksonia* smooth spore type. 2 = *Dicksonia* knobbled spore group. Data compiled from Collinson 1996, Lantz *et al.* 1999, Hill & Jordan 1998, Tidwell & Nishida 1993, Tidwell & Ash 1994.

1.6 Aims

General

- To evaluate the suitability of molecular data (e.g. from *rbcL*, *18S*, AFLP) for testing hypotheses on the evolution of tree ferns.

Tree Fern Clade

- Attempt to resolve relationships among taxa using *rbcL* gene sequences.
- To identify the main phylogenetic features of the tree fern clade.

Dicksonia

- To determine the affiliations of the New Zealand species to other members of the genus.
- To estimate the antiquity of the New Zealand species.
- To investigate the two observed growth forms of the NZ species *D. lanata* in terms of species separation.
- To determine the number and direction of dispersal events giving rise to the New Zealand species.

Cyatheaceae

- To investigate affiliations among NZ species.
- To investigate the relationship of *Hymenophyllopsis* to the Cyatheaceae.
- To attempt to determine the approximate age of the New Zealand species.
- To investigate the origins of the New Zealand Cyatheaceae and to estimate the number and direction of dispersal events bringing them to New Zealand.

MATERIALS & METHODS

2.1 Sampling

2.1.1 *Dicksonia*

Native species

Samples of *Dicksonia lanata* representing both growth forms were obtained from wild populations around New Zealand, covering most known sites in the North Island (see Figure 2.1). Exceptions were Taranaki, Great Barrier Island, Wellington and the South Island. Herbarium vouchers existed for these localities, but plants were either not found or no longer existed. South Island populations were not essential as their presence in the data matrix would be unlikely to affect the results, being furthest from the boundary of the two forms. Samples were taken in the form of 2 - 3 small pieces of frond tissue, approximately 10cm from the end of the frond. Soft, young fronds were selected preferentially as these were easier to extract DNA from. Frond tissue was placed in a plastic zip-lock bag to be taken to the lab for immediate extraction, or silica gel (6-18 mesh, BDH) was added for long term storage.

Samples of the other native *Dicksonia* species, *D. squarrosa* and *D. fibrosa* were collected from wild populations or from cultivated material, and stored as with *D. lanata* samples. Voucher specimens have been lodged at the Massey University Herbarium (MPN, see Appendix 2.1 for a complete list).

Foreign species

Samples of foreign *Dicksonia* species were obtained from overseas, or from cultivated material in NZ. Voucher specimens for these species are at the Massey University Herbarium (MPN). *Dicksonia antarctica* from N.S.W. was collected at Burraga Swamp in the Barrington Mountains by P.J. Lockhart and stored in silica gel. Other *D. antarctica* samples from New South Wales, and the Blue Mountains in Victoria

were sent from the Royal Botanic Gardens in Sydney, Australia. Three samples of *D. youngiae* were obtained from the Fern Society in Nelson, NZ. *Dicksonia sellowiana* was sent from the Royal Botanic Gardens in Sydney, Australia, from a plant originally from Chile. A second *D. sellowinana* sample (labelled as *D. berteriana*) was collected from a plant in Chile by Carlos Lehenbeck. *Dicksonia baudouinii* from New Caledonia was sent by Ewen Cameron.

2.1.2 Cyatheaceae

Native species

A sample of *Alsophila tricolor* was taken from an individual growing in the wild alongside the Ohakune Road and placed into silica gel. *A. smithii* was collected from the wild at Tongariro by M. F. Large. *Alsophila cunninghamii*, *A. milnei* and *A. kermadecensis* were sent from the University of Auckland, in the form of fresh pieces of frond in plastic zip-lock bags with moist tissue paper. *A. colensoi* was collected from the wild near Tongariro National Park. Fresh *S. medullaris* tissue was obtained from an individual growing on the Massey Campus at the back of the Computing Services building.

Foreign species

Samples of *Sphaeropteris robusta* and *S. excelsa* were sent from the Royal Botanic Gardens, Sydney, Australia. *Sphaeropteris tomentossima*, *Cyathea delgadii* and *Cyathea revolutum* were sent by Sandra Van der Mast from the Trigg Road Fern Nursery.

2.1.3 Other tree ferns

A sample of fresh *Lophosoria quadripinnata* tissue was sent by Dr. B. S. Parris, from a culitvated individual originally from Mexico. In addition to this was a sample of fresh *Cibotium glaucum*, from a cultivated individual originally from Hawaii. Vouchers were lodged at the Massey University Herbarium (MPN).

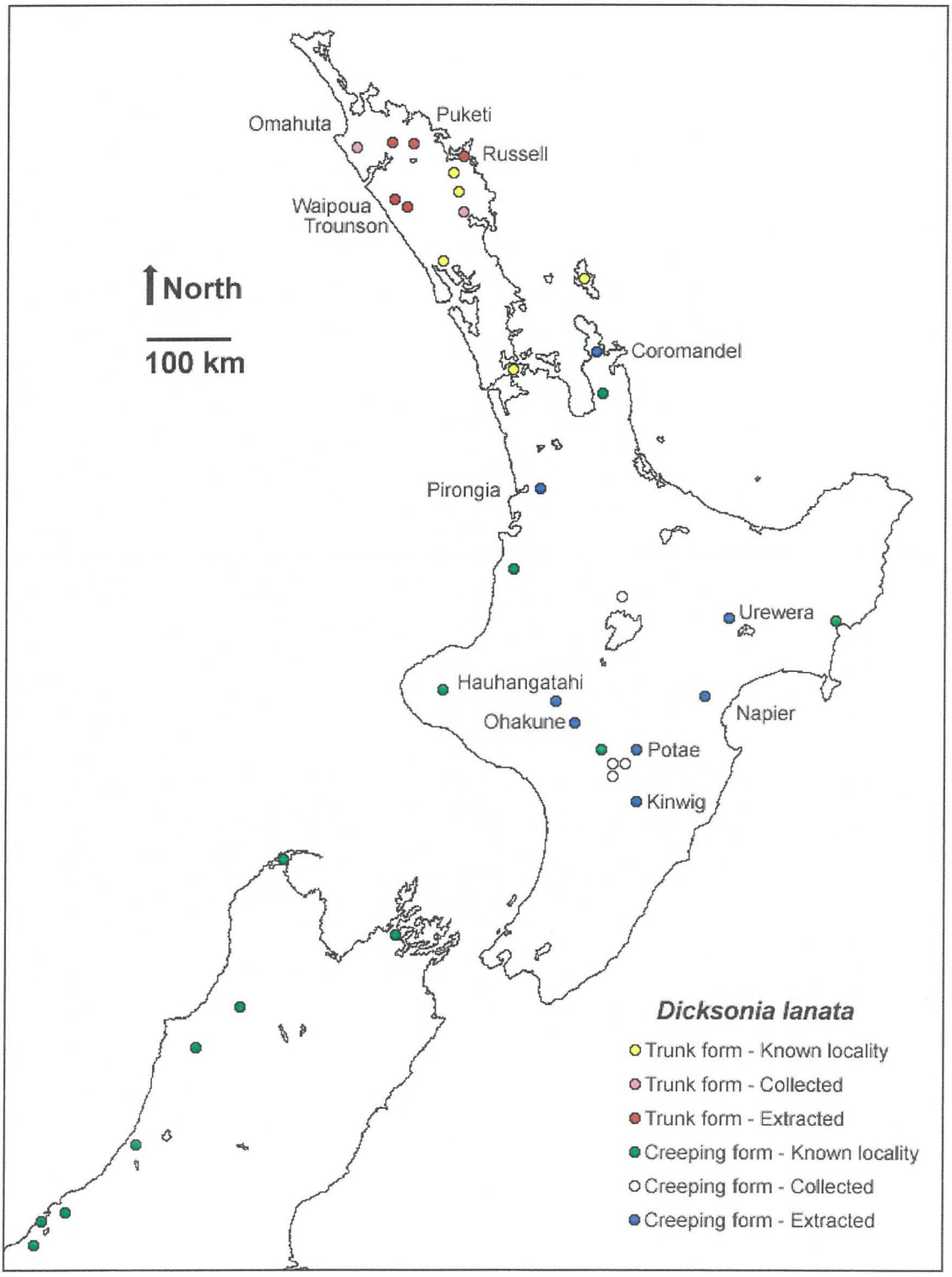

Figure 2.1 Distribution of *Dicksonia lanata*: "Known locality" indicates *D. lanata* population known from Herbarium vouchers (yellow/green). "Collected" indicates populations collected but not extracted (pink/white). "Extracted" refers to populations extracted and used in AFLP analysis (red/blue, labelled).

2.1.4 DNA Sequences

Existing *rbcL* and *18S rDNA* sequences were obtained from GenBank (www.ncbi.nlm.nih.gov). These include all existing *rbcL* sequences for both the Dicksoniaceae and Cyatheaceae, as well as sequences from the other families within the tree fern clade. An *rbcL* sequence from *Azolla caroliniana* Willdenow was also selected for use as an outgroup, as it is considered to be one of the closer relatives of the tree fern clade based on other *rbcL* studies (e.g. Hasebe *et al.* 1995).

Only one *18S* sequence was available within the tree fern clade, a sequence from *Dicksonia antarctica*. A sequence from *Adiantum raddianum* C. Presl was also selected for use as an outgroup, as this fern falls outside the tree fern clade, but yet is relatively closely related in reconstructed graphs (e.g. Hasebe *et al.* 1995).

2.2 DNA Extraction

Samples were extracted using a CTAB extraction procedure modified from Doyle and Doyle (1990). First, small fragments (~ 0.1 to 0.2 g) of fern tissue were placed in a 1.6 mL microfuge tube (Axygen Scientific) using sterile tweezers. The tissue was then crushed to a fine powder. This was accomplished by repeatedly freezing the plant tissue in liquid nitrogen and grinding it up with a glass rod.

Next, 600 µL of 2% w/v CTAB solution (see Appendix 2.2) was pipetted into the tube and mixed by shaking. The CTAB acts to help solubilise membranes, but most importantly its has a differential solubility for polysaccharides and nucleic acids at different salt concentrations. The CTAB buffer used contained 1.5 M NaCl. At concentrations above 0.7 M nucleic acids are soluble and polysaccharides insoluble. Its use under these conditions allowed isolation of fern DNA from cellular polysaccharides. Further, fern tissues generally contain large amounts of phenolic compounds, which can interfere with DNA extraction (Doyle & Doyle 1990). To remove this, polyvinylpyrollidine (PVP), at 1% w/v, was included in the extraction

buffer. The tube was then placed into a 65 °C heating block for approximately 40 min, to kill any nucleases and allow sufficient time for solubilisation of membranes.

After 40 min heating, 600 µL of chloroform was added, and the tube was shaken thoroughly to ensure a homogeneous solution. This was then spun briefly at 10,000 rpm in a microcentrifuge. The result was 2 layers of solution with the ground tissue compacted at the interphase. The top phase containing the DNA was taken off using a 1mL pipette and placed into an empty tube. At the interphase of the top and bottom layers remained the precipitated polysaccharides and other cellular debris.

To the DNA layer, 600 µL of isopropanol was added and the tube was inverted once and placed on ice. If the extraction was successful a white top layer and brownish bottom layer would form. After a while (a few seconds to several minutes) a ring of precipitated DNA would appear just below the interphase. This DNA was then taken out using a pipette, with the tip cut off a few mm from the end to reduce shearing of the DNA, and placed into a new tube containing approximately 1mL of 80% ethanol. This was then gently inverted a few times to wash the DNA. This wash (which removed residual polysaccharides, pigments and proteins) was repeated 2-3 times until the DNA was clean (white).

Extracted DNA was either stored in 80% ethanol at -20 °C, or the DNA was allowed to sink to the bottom of the tube (or gently spun down if it remained floating) and the ethanol was poured off. The samples were then air dried and resuspended in 40 µL TE (Tris-EDTA, see Appendix 2.2) buffer (or more if DNA was particularly concentrated). Samples in TE were stored at 4 °C overnight, then 3 µL was used to estimate DNA concentration, while the rest was stored at -80 °C.

DNA concentration was estimated by adding 1 µL of 5% RNAse in 10% loading dye (see Appendix 2.2) to the 3 µL of DNA in a 1.6 mL microfuge tube. This was then heated at 65 °C for 2 minutes to allow the RNAse to degrade any RNA present, and placed on ice. These DNA samples were then loaded onto a 1% Seakem LE agarose (FMC Bioproducts) gel in a 1X TAE buffer solution (see Appendix 2.2), with a 1 kb (kilobase, 1000 base-pairs) DNA ladder (Gibco BRL), and run at 100 Volts for 45

minutes to an hour. Agarose gels were then stained by soaking them in ethidium bromide for 15 - 30 minutes, and visualised over a UV transilluminator (wavelength 302 nm, UVP Incorporated). DNA concentration was estimated by rough comparison with the DNA ladder.

All DNA was stored at -80 °C. DNA in TE buffer was ready for use in PCR reactions and AFLP.

2.3 DNA Sequencing Markers and PCR

Commonly used DNA sequence markers were utilised to provide data for much of this study. An expectation from previous studies using these markers was that differences among the sequences should be useful for inferring evolutionary relationships amongst the tree ferns (e.g. Hasebe *et al.* 1995, Baldwin *et al.* 1995, Wolf *et al.* 1996, Taberlet *et al.* 1991, Nadot *et al.* 1995). For phylogenetic analysis both a chloroplast and a nuclear DNA sequence marker were chosen. Comparison between the results from the two genomes would hopefully uncover any systematic biases in the data and lead to better estimates of tree fern phylogeny.

2.3.1 Chloroplast DNA markers

rbcL

The *rbcL* gene is located on the chloroplast genome. It codes for the large subunit of the ribulose 1,5-bisphosphate carboxylase oxygenase enzyme (rubisco). The coding region is typically 1206 bp (base-pairs) in length. Comparison of the sequences from different species of plants has been widely used to infer phylogenetic relationships (e.g. Chase *et al.* 1994), and has been used successfully in ferns (e.g. Hasebe *et al.* 1995). The *rbcL* gene is useful for this purpose as it has a slow synonymous mutation rate compared to nuclear genes, and functional constraints that limit the evolutionary rate of nonsynonymous substitutions (Hasebe *et al.* 1994). These factors result in the sequence of the gene being well conserved among taxa and useful for higher level

phylogenies. In this study, 7 primers were used in *rbcL* sequencing. Primers 'aF' and 'cR' were taken directly from Hasebe *et al.* (1994). A further four primers, named '422F', '961F', '579R' and '988R', were designed by L. R. Perrie for use in *Polystichum*. One of the primers was redesigned after sequencing difficulties in some taxa, and was named '579Rdic'. The sequences of all primers used may be found in Appendix 2.3.

For *rbcL* PCR the aF and cR primers were used. These were run using the TABER program (Appendix 2.3) with the extension time increased to 1'30", and the annealing temperature raised to 60 °C.

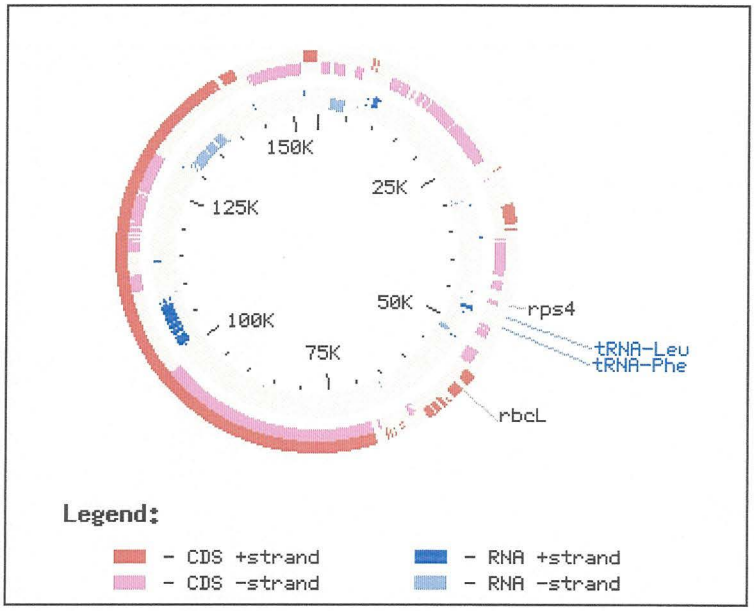

Figure 2.2 *Arabidopsis thaliana* Chloroplast Map: Shows the chloroplast markers utilised in this study and their relative positions in the *Arabidopsis thaliana* chloroplast genome. tRNA-Leu and tRNA-Phe indicate the approximate position of the *trnL-trnF* spacer and *trnL* intron regions. Modified from www.ncbi.nlm.nih.gov/.

rps4

The *rps4* gene encodes protein 4 of the chloroplast ribosomal small sub-unit. It was first employed as a phylogenetic marker by Nadot *et al.* (1994). The gene is short, approximately 600 bp in length, and being a coding sequence, is well conserved. It has been sequenced successfully for a range of taxa, including ferns (Nadot *et al.* 1995, L. R. Perrie pers. comm.). At the 3' end of the gene is a spacer region followed by the chloroplast *trnS* gene. In this study primers were used to amplify the *rps4* gene

and the spacer region following it. The primers used were labelled rps4F, rps2 (from Nadot *et al.* 1994) and trnSR (designed by L. R. Perrie from *Polystichum* sequence).

Initially the primers rps4F and trnS were used for *rps4* amplification. However, these failed to amplify a single product and the internal primer rps2 was tried (with trnS). PCR reactions were run on a PCR machine using the RPS4 program (see Appendix 2.3).

trnL-trnF spacer and trnL intron

The *trnL-trnF* spacer and *trnL* intron are two of three non-coding region chloroplast markers described by Taberlet *et al.* (1991). The *trnL* intron is found within the chloroplast *trnL* gene, while the *trnL-trnF* spacer separates this gene from the *trnF* gene. Both regions vary in length, but both are short. The *trnL* intron is around 600 bp in length, the *trnL-trnF* spacer only a few hundred base pairs. They have been successfully used for phylogenetic analysis in a range of plant groups at inter- and intra-specific levels (e.g. Potter *et al.* 2000), including ferns (L. R. Perrie pers. comm.). Both are more variable than *rbcL* due to less functional constraints, and prone to length mutations (insertions or deletions). It has also been noted that long series of A's and T's may be present in the sequence of the *trnL-trnF* spacer may make it difficult to read and align (Gielly & Taberlet 1994). Due to difficulties in aligning the sequences of more diverse species, the *trnL-trnF* spacer is recommended by these authors for use in intrageneric studies. It can be amplified using two primers originally described in Taberlet *et al.* (1991), labelled simply 'e' and 'f'. In this study the primers were designated 'TabE' and 'TabF' (after Taberlet). The *trnL* intron is amplified by the primers 'c' and 'd', labelled 'TabC' and 'TabD' in this study. Primer sequences are given in Appendix 2.3.

To amplify the *trnL-trnF* spacer region the primers TabE and TabF were used. For the *trnL* intron the primers TabC and TabD were tried initially. If amplification was unsuccessful TabC and TabF were used in combination to amplify both regions simultaneously. Thermocycling used the TABER program (see Appendix 2.3).

2.3.2 Nuclear DNA markers

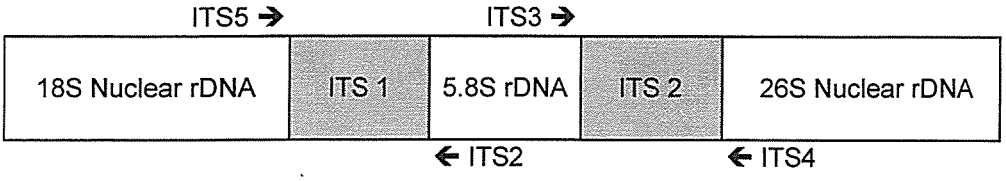

Figure 2.3 The Nuclear rDNA Region: This region is a repeated unit with multiple copies in the nuclear genome, coding for the RNA components of the ribosome. The *18S* and *ITS* regions were used in this study. Modified from Baldwin *et al.* 1995.

ITS

The *ITS* region is located in the nuclear genome, and describes the internal transcribed spacer region of the ribosomal RNA genes (Baldwin *et al.* 1995). There are two spacers, ITS-1 and ITS-2 which lie either side of the 5.8S rRNA gene. Each is about 300 nucleotides in length. Although present in multiple copies in the genome the copies often have the same sequence due to mechanisms of gene conversion. The sequences of the spacers are relatively fast evolving, which makes them useful for studying lower level evolutionary relationships (e.g. at genus, species level and in some cases intra-specific level, Lockhart *et al.* 2001). Four primers were used to attempt to amplify this region, the external primers 'ITS5' at the 5' end, and 'ITS4' at the 3' end, and the internal 'ITS3' and 'ITS2' primers. The sequences of these may be found in Appendix 2.3.

In *ITS* amplification the primer pair ITS4 and ITS5 was first used, and in some cases also the pair ITS2 and ITS5. These reactions were run using the ITS program (Appendix 2.3).

18S

The nuclear 18S ribosomal DNA is a very highly conserved gene coding for the 18S ribosomal RNA molecule, which is one of the major RNA's found in the ribosome. It is approximately 1.8 kb in length, and is present in multiple copies in the nuclear genome, as part of a ribosomal DNA unit which also contains genes for the 5.8S rRNA and 26S rRNA (see figure 1 in Baldwin *et al.* 1995). Mutations within this gene

are highly likely to adversely affect the survival of any organism due to its ubiquitous role in the cell. This low mutation rate makes it a useful marker in the study of higher order relationships among organisms, and has been used to probe deep divergences, such as the phylogeny of land plants (Soltis *et al.* 1999). It was hoped that this marker might provide some phylogenetic signal due to the antiquity of the fern families being studied (e.g. early Cretaceous c. 140 mya divergence of Dicksoniaceae and Cyatheaceae). Four primers were used in this study, two were adapted from Medlin *et al.* (1988). These were their primers 'A' and 'B' (minus the linkers), here renamed as '18sF' and '18sR'. Two internal primers were designed from an alignment of previously determined *18S* sequences from ferns available from GenBank (www.ncbi.nlm.nih.gov). These were named '18sF2', and '18sR2'. Sequences of these primers can be found in Appendix 2.3.

For *18S* PCR the 18sF and 18sR primers were used. The TABER program was used to for thermocycling, with the extension time increased to 2 minutes.

2.3.3 PCR Reactions

Once selected, DNA markers were then amplified by PCR (the Polymerase Chain Reaction, e.g. Saiki *et al.* 1988) from extracted DNA stored in TE buffer. DNA was usually diluted to 1/100, but sometimes less dilute solutions were used if the DNA concentration of the sample was low. PCR reactions were set up by preparing a master PCR reaction cocktail (see Appendix 2.3), which was subsequently pipetted in 19 µL aliquots into labelled 200 µL PCR tubes (Sorenson Bioscience) and kept on ice prior to thermocycling. Use of a master mix ensured all samples had identical concentrations of reaction components. 1 µL of diluted DNA was added per tube, and one tube was always left without DNA as a negative control.

Reaction tubes were then placed into a PCR machine (either a PTC-200 (Peltier Thermal Cycler) DNA Engine, or a PTC-150 Minicycler, both from MJ Research). Thermocycling conditions consisted of three steps, annealing, extension and denaturing, repeated for c. 30 - 40 cycles. In the annealing step, reaction conditions favoured primers specifically binding to their target sequences. The annealing

temperature of the primer was the most variable factor, and was increased to eliminate non-specific binding (mis-priming), or reduced to increase the yield of amplified product, as required. In the extension step, the DNA was copied by the DNA polymerase, at 72 °C (optimal for Taq polymerase), for a time period dependent on the length of the target sequence (approximately 1 minute per 1 kb). Finally, the denaturing step, at 94 °C, separated all DNA strands, ready for another cycle. Each cycle approximately doubled the number of marker sequences present, with an exponential increase over the 30 - 40 cycles.

After PCR samples were kept at 4 °C. A 3 µL sub-sample from each tube (including the negative control) was taken to check for amplification. These were combined with 1 µL of 10X loading dye and loaded on to a 1% agarose gel, along with 10 µL of 1 kb DNA ladder. The gel was run at 100 Volts for 45 - 60 minutes, stained with ethidium bromide, and visualised over a UV light. Presence of a single, bright band of the desired size was indicative of successful amplification. If multiple bands were evident in any samples, the PCR was repeated, using an increased annealing temperature (generally 3 °C). Bands in the negative control indicated contamination and all samples were discarded.

PCR products from successful reactions were separated from unincorporated primers, oils, salts and proteins using a Life Technologies Concert Kit. The Life Technologies protocol was followed with the substitution of 40 µL of TE buffer instead of 50 µL, to give more concentrated products. Samples were also left for 5 minutes after the TE was added instead of 1 minute (again to increase yield). Once cleaned, PCR products in TE buffer were checked for concentration on agarose gels against a Low DNA Mass Ladder (Gibco BRL). 3 µL from each sample was mixed with 1 µL of 10X loading dye. In addition, 2 µL of Low DNA Mass Ladder (Gibco BRL) was mixed with 1 µL of loading dye. These solutions were loaded onto a 1% agarose gel, along with 10µL of 1 kb DNA ladder. Gels were run and stained as before. DNA concentration was estimated by comparison of band brightness with bands in the Low DNA Mass Ladder.

Cleaned PCR products were stored at -20 °C until required for sequencing.

2.3.4 Sequencing Reactions

Cleaned marker fragments were then sequenced using an automated fluorescence sequencer. This procedure uses a different fluorescent label for each of the four bases, A, G, C and T. The PCR products are run in a special PCR reaction with only a single primer, and a mixture including a majority of unlabelled dNTPs with a smaller ratio of the labelled dNTPs. Because only a single primer is used only one strand will be sequenced per sequencing reaction. Extension continues along from the primer as normal until one of the labelled dNTPs is incorporated into the newly synthesised DNA strand. At this point the extension stops, resulting in a complementary strand with a terminal fluorescently labelled base. It is expected that over a large number of cycles, each base in the DNA sequence will be represented by a number of fluorescently labelled copies. Once these PCR reactions (called Autosequencing reactions) are complete the labelled products are precipitated out of the solution and then loaded onto the Automatic Sequencer. The products are then run by electrophoresis, which migrate according to size, with smaller fragments travelling faster than larger ones. These move past a laser detector which identifies each of the fluorescent labels as it passes. Output is recorded by computer which results in a plot of peaks for each of the different labels, which correspond to individual bases in the DNA sequence (referred to as an electrophoretogram). Of course, this sequence will be the reverse-complement of the DNA strand being sequenced, but this can easily be flipped by computer to give an electrophoretogram which represents the sequence of the opposite strand.

Sequencing reactions were performed using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer). These were set up in labelled 200 µL PCR tubes, including 4 µL of BigDye Terminator (Perkin-Elmer) reaction mix, 1.6 µL of primer (1.6 pmol), and DNA in the range of 45 - 90 pg / 25-45 ng (usually 3 µL). The reaction was made up to a total volume of 10 µL with H_2O (usually 1.4 µL). These tubes were then placed in a PCR machine and the AUTOSEQ program was run (see appendix 2.3). Sequencing reactions were performed using primers on both strands of the DNA so that sequences from both strands were obtained. These could

be compared and any discrepancies checked, increasing the reliability of the final sequences.

For *A. tricolor, A. milnei, A. cunninghamii* and *A. kermadecensis*, the presence of mononucleotide repeats in the DNA sequence of the *trnL-trnF* locus resulted in poor sequencing (discussed further in Section 5.1.2). In attempt to counter this two things were tried, separately and in combination. First, the denaturation step of the sequencing reactions was increased to 1 minute and the annealing step to 30 seconds. Secondly, reactions were set up using dGTP reaction mix (Perkin-Elmer) rather than the BigDye Terminator mix. This reaction mix is designed to improve sequencing of templates with mononucleotide repeats of C's.

Sequencing Product Precipitation

After the sequencing reactions were run the reaction products were precipitated using a sodium acetate procedure. To the tubes containing the sequencing reactions 1µL of 3M sodium acetate (pH 5.2) was added. These were then transferred to labelled 1.6mL eppendorf tubes. 25 µL of chilled 100% ethanol was then added to these and they were left on ice for 15 minutes. After 15 minutes the tubes were centrifuged at 14,000 rpm at 4 °C for 15 minutes. The supernatant was then taken off using a 200 µL pipette and 500-750 µL of chilled 80% ethanol was added. The tubes were then inverted a few times and centrifuged again at 14,000 rpm at 4 °C for a further 15 minutes. The supernatant was then tipped off and the tubes were air dried until no ethanol remained. The tubes were then stored at -20 °C until sequenced.

Sequencing was carried the Massey University DNA sequencing facility (MUSEQ) by Lorraine Berry on an Applied Biosystems 373A DNA Sequencing System.

2.3.5 Sequence Editing

Sequences returned from MUSEQ were in the form of electrophoretograms, graphs plotting the sequence of bases along the DNA strand, with peaks of a different colour corresponding to each of the four separate bases. These files were analysed on an

Apple iMac computer using the MT Navigator PPC (ABI Prism) program. For the first sequence returned from each marker, some of the sequence was pasted into a BLAST query form on the NCBI home page and checked against other sequences in the database. Matching sequences from the same marker in other species were interpreted as indicative of successful target sequencing. Then, in MT Navigator, sequences from both strands of the DNA were compared directly by changing the reverse strand into the same orientation as the forward strand using the 'reverse complement sequence' function. These two sequences were then copied from MT Navigator into a text file and aligned by eye. Any discrepancy between the sequence from the two strands was checked on the electrophoretograms. DNA sequence from the two strands were edited to produce a final sequence in the 5' direction for each species-marker combination.

2.4 AFLP

Amplified Fragment Length Polymorphism, or AFLP, is a DNA fingerprinting technique first published by Vos *et al.* (1995). It is now frequently used in the study of genetic variation within populations (e.g. Perrie *et al.* 2000, Drummond *et al.* 2000). In this study it was used to assay genetic variation between *Dicksonia lanata* populations.

The technique is a combination of RFLP (Restriction Fragment Length Polymorphism) and PCR techniques. No prior knowledge of the genomes being studied is required. Using generic primers a proportion of DNA fragments cleaved by restriction endonucleases are amplified by PCR to give a banding pattern, or fingerprint. These patterns are typically visualised on polyacrylamide or agarose gels using silver staining or fluorescent dyes. The large number of bands that can be produced by using this approach can provide useful information for distinguishing closely related taxa. However, it is generally unknown whether bands of the same size are homologous. Further, there may be a lack of independence between some bands, and both problems can reduce the usefulness of fingerprint data in phylogenetic studies (Swofford *et al.* 1996)

2.4.1 Restriction Digest

The first step in generating an AFLP gel is to digest genomic DNA using restriction enzymes. These enzymes cut the DNA at specific target sequences which occur randomly throughout the genome. Generally a rare cutter and a frequent cutter are used in combination. In this study the restriction enzymes EcoRI and MseI (both from New England Biolabs) were used. EcoRI is a rare cutter, with a target sequence 6 bp long, and will cut on average every 4 kb. The MseI enzyme is a frequent cutter, with a target sequence only 4 bp long. MseI will produce generally smaller fragments, cutting on average every 250 bp. Using them in combination results in three types of fragment. First, ones cut with MseI at both ends will be the most common, with much smaller numbers of fragments which have an MseI cut at one end, and an EcoRI cut at the other. The third class has EcoRI cuts at both ends, and will be the least common.

Restriction digests of genomic DNA were set up in labelled 1.6 mL microfuge tubes. A 25µL total volume was prepared with 5 µL of 5X Reaction buffer (Gibco BRL), 1 µL of EcoRI and 1 µL of MseI enzymes, and 1-5 µL DNA (depending on DNA concentration in the sample). H_2O was added to make the reactions up to 25 µL. Reactions were incubated at 37 °C for 3 hours, then placed in a 70 °C heating block for 15 minutes to denature the enzymes. After this they were placed on ice at 4 °C for 20 minutes and spun down gently. 5µL of restricted DNA (with 1 µL of 10X dye added) was run on an agarose gel to check that digestion had occurred. An even intensity smear on the gel with no high molecular weight band (at approximately 20 kb) was taken as indicative of a good digest.

2.4.2 Ligations

Once the genomic DNA had been digested linkers were added to the ends of the DNA fragments. Restriction enzymes such as EcoRI and MseI produce single stranded 'sticky ends' when the DNA is cut. To produce double stranded DNA, linkers are added to the ends of the fragments. These are short pieces of DNA with single stranded overhangs complementary to those of the restriction fragments. Linkers were

attached to the DNA by T4 ligase which catalysed covalent bonds between the linker and restriction fragment. This ligation produces double stranded DNA at both ends of the restriction fragments, which were then used as priming sites for PCR amplification.

The ligation reactions were set up in labelled 1.6 mL microfuge tubes. A 10 µL total volume was prepared with 2 µL of 5X T4 Ligation buffer (Gibco BRL), 0.5 µL of T4 DNA Ligase (Gibco BRL), 0.5 µL of EcoRI Linker, 0.5 µL of MseI Linker, and 4 - 6 µL Restricted DNA (mostly 5 µL, but 4 if sample appeared strong, 6 if weak). H_2O was added to make up to 10 µL. Reactions were incubated overnight at 4 °C, then stored at -80 °C.

2.4.3 PCR steps

After ligation, the digested genomic DNA was present as fragments of varying length, the ends of which can be targeted by PCR primers (as the restriction site and linker sequences are known). PCR conditions were used so that typically only restriction fragments 0.5 – 1 kb are amplified. Many of these have an MseI cut at one end and an EcoRI cut at the other end. For complex genomes (such as plant genomes) the number of amplified bands is large. Thus a second selective PCR step is used to reduce the complexity of the profile.

Pre-amplification

The pre-amp reaction uses primers that anneal to the linker and restriction site plus one additional nucleotide which is arbitrarily chosen. This will target a fraction of the total fragments present. In this study EcoRI + A and MseI + C were used for pre-amp reactions. In this example, of the MseI ends, approximately 1/4 will match the additional 'C' of the primer, and similarly approximately 1/4 of the EcoRI ends will match the extra 'A'. This will result in roughly 1/16 of the total EcoRI-MseI fragments being amplified.

Pre-amp reactions were set up by preparing a master reaction cocktail (see Appendix 2.4) which was then aliquoted out into labelled 200 µL PCR tubes (19 µL per tube) and kept on ice. To each sample 1 µL of ligated DNA from the previous step was added. PCR thermocycling conditions used are described in the AFLPPA program (Appendix 2.4).

Selective amplification

After the pre-amp a final selective amplification was performed. This involved amplification of the pre-amp products with a primer like that used in the pre-amp, but with a further two nucleotides added. Selective EcoRI primers with additional base that were A-T rich seemed to give better results in previous fern work (L.R. Perrie - pers comm), thus these were also used in the present study.

Selective amplification reactions were set up again by preparing a master reaction cocktail (see Appendix 2.4), which was pipetted out in 19 µL aliquots into labelled 200 µL PCR tubes, which were kept on ice. Pre-amp DNA was diluted to 1/50 in labelled 1.6 mL microfuge tubes, from which 1 µL was taken out and added to the selective amplification tubes. Pre-amps could be retained and used for multiple selective amplifications. However, after repeated freezing and thawing they tended to break down. Thermocycling conditions used the AFLPSO program (Appendix 2.4) on a DNA engine PCR machine (MJ Research PTC-200 Peltier Thermal Cycler).

After selective amplification, 8ul of DNA was run on a 3% agarose gel to check that previous steps had been successful before running them on a polyacrylamide gel. A smear of DNA, with some discernible bands was interpreted as indicative of successful selective amplification.

2.4.4 Polyacrylamide Gel Electrophoresis

The final stage of the process was to run the PCR products out on an 8% polyacrylamide gel, which is a gel matrix that has much better resolution than agarose.

The gel is poured between two glass plates, one of which is treated so that the gel will bond to it, the other so that it will not. Gel plates were first prepared by washing them with soap and water and then air dried. To the short plate a solution of bind-silane was added (2 mL 95% ethanol, 10 µL Acetic Acid and 1 µL of Bind-silane, Pharmarcia). This facilitates the formation of covalent bonds between the glass plate and the polyacrylamide gel. This was spread evenly over the glass plate with a tissue and allowed to dry for c. 5 minutes. The plate was then washed with four 2 mL aliquots of 95% ethanol to remove any excess bind-silane and allowed to dry. The long plate was treated with RAIN-X ®, as per the instructions on the bottle. This solution is water repellent, and helps to prevent the gel sticking to this plate.

The cleaned, treated plates were then placed on top of each other with thin (0.4 mm) strips of plastic placed along the edges to separate them, creating a space into which the gel could be poured. A rubber seal was then placed around the edges of the plates to seal them, leaving only the top edge unsealed.

The gel was made up first by dissolving 29.4 g of Urea in 7 mL of 10X TBE buffer (see Appendix 2.4) and about 45 mL of MilliQ H_2O in a glass beaker. This solution was then filtered using Whatman Filter Paper into a 100 mL measuring cylinder. Using extreme care 7 mL of Long Ranger Gel Solution (FMC Bioproducts) containing acrylamide was also filtered into the cyclinder. H_2O was added in to make the solution up to 70 mL. This was then poured into a glass beaker and 350 µL TMED (BDH) and 35 µL APS (10% ammonium persulphate, BDH) were added, to catalyse the polymerisation of the gel. The beaker was swirled briefly to mix the solutions.

Approximately 30 mL of the gel solution was then drawn into a 60 mL syringe, and the gel held at an angle, so that when the gel solution was applied to the top corner, it would flow down the side of the plastic spacer into and fill the space between the plates from the corner. When the syringe was empty the gel was gently laid down at a slight angle. More solution was added, moving across the top of the gel until the entire space between the plates was filled. Care was taken to avoid air bubbles in the gel. Plastic combs were placed upside down into the gap between the plates to create a

straight edge along the top of the gel, and bulldog clips were used to hold these in place. The gel was then allowed to set for at least 1 hour, then the top was wrapped with plastic to minimise drying of the gel, and it was kept overnight at 4 °C.

The gel was set up in an S2 gel rig (Gibco BRL). 1.2 L of 1% TBE buffer was prepared, and some was added to the upper portion of the rig and left for c. 5 minutes to check that it was watertight. If not, the TBE was allowed to drain out and the gel was set up again. TBE buffer was then added to the lower bay of the rig, so that the upper and lower edges of the gel were submerged. The gel was then pre-warmed at 55 W for 15 - 30 minutes, without samples.

Loading

3.5µL from each sample was added to 1.5µL of formamide dye in labelled 200 µL PCR tubes. The DNA was then denatured in a DNA engine PCR machine by heating to 94 °C for 4 minutes, followed by rapid cooling to 4 °C. These samples were then placed on ice, ready for loading.

Combs were placed between the plates along the upper edge of the gel to create approximately 48 wells. Each sample was then loaded into a separate well, using a flat-head pipette tip. 4µL of 100 bp ladder (Gibco BRL) was loaded on both sides of the samples. The gel was then run for approximately 2-3 hours at 40 W. If two sample sets were to be loaded, the gel was run for 1 hour and then stopped while the second set was loaded, and then run for a further c. 2 hours.

Once electrophoresis was complete, the glass plates were separated. The short plate (with the gel on it) was placed in 4 L of 10% acetic acid for 2 hours under gentle agitation. This removed the urea from the gel, which would otherwise interfere with the staining process.

The gels were stained by a silver nitrate staining process, in which silver ions were bound to the negatively charged DNA. These were developed in a manner similar to the development of photographs, to produce black bands wherever DNA was present

in the gel. First, the gel plate was then taken through three 5 - 10 minute washes in Milli-Q H_2O to remove the acetic acid. It was then placed in a staining solution of 3 g silver nitrate and 4.5 mL of formaldehyde in 3 L of Milli-Q H_2O for 1 hour. The gel plate was passed through a quick wash in chilled H_2O, before being placed in 2 L of developing solution (120 g sodium carbonate, 450 µL sodium sulphate and 6.5mL formaldehyde in 4 L H_2O) until bands began to develop. It was then placed in the remainder of the developing solution until the bands had fully developed. At this point the reaction was stopped by the addition of 2 L of 10% acetic acid (saved from the previous acetic acid wash). This was left until the reaction was complete (until no more bubbles formed on the bottom of the tray, not more than 2 - 5 minutes).

The developed gel was washed in RO H_2O to remove the acetic acid, then air dried overnight.

2.5 Phylogenetic Analysis

Raw data obtained from either DNA sequencing or AFLP was analysed using a number of computer programs including MT Navigator, ClustalX, PAUP* 4.0b5 and SplitsTree 2.4. These ultimately produced phylogenetic graphs, representing the relationships among the species or populations, from which phylogenetic inferences were drawn (see Sections 3.1, 4.1, 5.1).

2.5.1 Alignment

Typically phylogenetic analysis of sequence data requires the sequences to be first aligned as part of a multiple sequence alignment such that bases at a particular position in the alignment share a common ancestry. In some cases, particularly non-coding sequences, gaps may be present in some of the sequences after alignment, representing insertion or deletion of bases (indels). Alignment of DNA sequences can be accomplished by computer using progressive alignment programs such as ClustalX or simultaneous multiple alignment programs such as DCA. These programs assign particular weights, or scores, to different substitution types, i.e. transitions versus transversions, and then progressively align sequences sequentially. As it does this, it

introduces gaps into the sequences if these are necessary for maximising similarity. Progressive alignment with ClustalX uses a guide tree (Neighbor Joining) to determine the order that sequences or groups of sequences are aligned. Once gaps are introduced during this procedure their relative positions are not re-evaluated. Multiple alignments can become problematic for large sets of taxa, and/or for very diverse sequences, especially those of very different lengths.

The alignments generated by this approach are given in Appendices 3.1, 4.1 and 5.1.

2.5.2 AFLP Analysis

A light box was used to help visualise the gel. Bands were scored and coded as present or absent. Bands of the same size on the gel were assumed to indicate amplification of homologous PCR products. This assumption of homology presumably resulted in some noise in the data.

The binary coded data from the AFLP gels was formatted into a nexus file and studied under both SplitsTree 2.4 and PAUP* 4.0b5. In studying populations and the two forms of *Dicksonia lanata*, the *D. squarrosa* samples were used as outgroups.

2.5.3 Sequence Analysis

Sequences aligned by ClustalX were formatted into a nexus file. These were then studied under both SplitsTree 3.1 and PAUP* 4.0b5 (PPC). Evolutionary trees may be reconstructed by a variety of methods, but these can be recognised as involving either "algorithmic" or "global optimality" methods.

Algorithmic Methods

Algorithmic methods include Neighbour Joining (NJ) and UPGMA. These use an algorithm in order to cluster taxa into a bifurcating tree. The data analysed is in the form of a "distance matrix", a table of relative distances between each pair of taxa. In the present study, Neighbour Joining was used for reconstruction of evolutionary trees. It is preferable to UPGMA, as it does not assume a molecular clock. Both

methods start with a pairwise distance matrix. The values in the matrix can be either objective distance estimates or more general path length measures such as sequence dissimilarity. Taxa are progressively clustered into a bifurcating tree by using criteria that determine the order of clustering. UPGMA uses d_{ij} values, which are unadjusted distances. Neighbour Joining uses S_{ij} values, distances to which a correction has been applied. These methods are computationally very fast as only a single tree is built.

Global Optimality Methods

These methods typically evaluate and compare the overall fit of the data to fully resolved bifurcating trees. Tree selection criteria include maximum parsimony and maximum likelihood. While computationally slower than algorithmic methods they have the advantage of examining a large numbers of possible trees. Two different types of search strategy are available, "exact" and "heuristic". Exact searches may be either exhaustive, or branch and bound. An exhaustive search examines every possible tree to identify the best tree under the selected optimality criterion. This is impractical for sets of taxa greater than c. 10, due to the exponential increase in possible trees to be evaluated when additional taxa are added. A branch and bound search eliminates subsets of possible trees that exceed the score for the current best tree. Addition of further taxa to the sub-optimal tree is discontinued, reducing the number of trees that need to be examined. This method should retrieve the optimal tree for the data without requiring the computational power of an exhaustive search. In practice up to 20 taxa may be evaluated in this manner. For greater numbers of taxa heuristic searches are required. These do not examine the scores for all possible trees. Rather, strategies are employed which have empirically been found to find optima. Heuristic searches have the advantage of being fast, but they may not select the optimal tree for the data.

Under the maximum parsimony (MP) criterion the best tree(s) will simply be the one(s) that require the least number of changes to fit the data. With highly divergent sequences, dissimilarity measures will underestimate the total number of substitutions between sequences. In these cases corrections for multiple base changes, using an explicit stochastic model, are required.

Maximum Likelihood (ML) calculates the probability that a given tree evolved the data, under a given model of sequence evolution. Models range in complexity from those incorporating a single rate for all nucleotide changes (Jukes-Cantor,1969), to those estimating sequence base and amino acid compositions and rates classes for different sequence positions. The most appropriate model for a given data set can be evaluated using a "likelihood ratio test", in which log likelihood scores for each model are compared.

Quartet Methods

These methods evaluate support for the 3 possible bifurcating trees on combinations of 4 taxa. Quartet puzzling uses the best quartets in a heuristic approach to build a tree. Split decomposition identifies the two best supported trees for every quartet and uses this information to define splits and build a network.

Split Decomposition (Huson 1998) can be employed to examine the assumption that data fit a bifurcating evolutionary tree. In this method each quartet is generally evaluated by a distance criterion (although the option of using parsimony is also available), and the two best unrooted trees are retained to build a subsequent split system. These are said to be "weakly compatible", hence split decomposition may be described as the sum of weakly compatible splits. As some quartets identify the same split between taxa it must be decided how to determine the value of this split in the final split system. SplitsTree 3.1 uses a conservative approach, and assigns the smallest value to the split. This may result in internal edges shrinking or collapsing when there are highly diverged sequences in the data matrix. Least squares edge fitting may be used to optimise the edge lengths within the graph if the edges have not been collapsed to zero. However, it will not recover splits that have collapsed to zero. Once all splits have been identified and assigned values a graph is constructed to represent this split system. If no incompatibility is present the resulting graph will be tree-like. If conflict is present within the data this will result in reticulation in the graph (producing a box-like network). Tree-like graphs showing little conflict indicate that data is appropriate for use in building a bifurcating tree. A fit statistic returned for each splits graph gives an indication of how well the data is represented by the graph. This number ranges from 0 to 100.

Quartet Puzzling (QP, Strimmer & von Haeseler, 1996) is another quartet method, which generates a bifurcating evolutionary tree. In this method all possible quartets are evaluated under a selected optimality criterion (distance, parsimony or maximum likelihood) and the best supported unrooted tree for each is used in subsequent tree building. A global heuristic search is then used in a large number of "puzzling steps" (usually 1000) to produce a bifurcating tree. Puzzle values for groupings of taxa give an indication of support for relationships within this tree, and are usually high when sequence data shows few informative sites but also few incompatibilities. This gives an advantage over non parameteric bootstrap values, which tend to be low under similar conditions.

Non parametric bootstrapping involves re-sampling the data matrix to produce 100 or 1000 etc. new data matrices. A tree is built from each one, and the number of times taxon *i* groups with taxon *j* in the 100 or 1000 trees etc. is recorded. High bootstrap values result from the relatively high frequency of pattern types that favour taxa being closely related under the tree selection criteria used. Low bootstrap values can occur because there is conflict in the data or because, relative to the sequence length, there are few informative sites.

Molecular Clock Analysis

The term "molecular clock" implies that different lineages evolve at a constant rate over time. The occurrence of a molecular clock may be useful in phylogenetic analysis, as its assumption permits midpoint rooting of evolutionary trees. To test for the fit to a molecular clock of a given set of taxa a "likelihood ratio test" may be performed. Trees generated in PAUP* can be described using maximum likelihood with and without a molecular clock assumed, giving log likelihood values for each. A ΔW value is then calculated as 2 (*ln* likelihood clock – *ln* likelihood no clock). This value is then compared with the χ^2 value with (n-2) degrees of freedom (where n is the number of sequences) to test for significance. If the ΔW is less than the χ^2 value then a molecular clock may be assumed. As the assumption of a molecular clock

requires a rooted tree, alternative root positions were evaluated for their fit to a clock. This test was performed on all markers sequenced in this study.

TREE FERN CLADE

3.1 Results

Results from phylogenetic analysis of the *rbcL* gene are reported in this chapter. Sequences for this chloroplast region have been characterised in a wide range of tree fern genera, thus their analysis provides an overview of the genetic relationships within the tree fern clade. Details of DNA extraction, PCR and sequencing of the *rbcL* gene, and have been given in chapters 4 and 5, and methods are described in chapter 2.

3.1.1 *rbcL* Sequences

rbcL sequences that we determined as part of this study were compared with seventeen *rbcL* sequences retrieved from GenBank (accessed through the NCBI home page at www.ncbi.nlm.nih.gov). Sequences for two *Alsophila,* two *Sphaeropteris* and six *Dicksonia* species were determined in this study (details on the PCR and sequencing of these species have been given in Chapters 4 and 5). Two additional *Dicksonia* sequences (*D. lanata, D. squarrosa*) were available from unpublished work by L. R. Perrie. A total of twenty-nine *rbcL* sequences were studied.

3.1.2 DNA Sequence Alignment

The coding region of the *rbcL* gene in tree ferns is 1320 bp in length. Positional homology between these and outgroups was inferred using ClustalX. Use of a multiple alignment procedure was necessary because some sequences were not complete, e.g. *D. lanata, D. squarrosa.* Also, the published sequence from *Azolla caroliniana* was 12 bp longer at the 5' end, while the *Lophosoria quadripinnata* sequence had an extra 2 bp of sequence at the 3' end. Summary statistics are given in Table 3.1 and the full alignment may be found in Appendix 3.1.

Marker + Taxa	No. Seq.	Length Range	Aligned Length	Single bp changes	Percent Variability	Base Composition A	C	G	T	% A-T
***rbcL* (chloroplast)**										
Dicksoniaceae[1]	15	1230 - 1320	1334	136	10.2%	0.250	0.225	0.189	0.336	58.6
Dicksoniaceae+*Lophosoria*	16	1230 - 1322	1334	138	10.3%	0.246	0.228	0.194	0.332	57.8
Cyatheaceae[2]	7	1265 - 1320	1334	45	3.4%	0.216	0.283	0.197	0.305	52.1
Cyatheaceae+*Hymenophyllopsis*	9	1265 - 1321	1334	67	5.0%	0.234	0.302	0.169	0.295	52.9
Dicksoniaceae+Cyatheaceae[3]	25	1265 - 1322	1334	191	14.3%	0.245	0.246	0.190	0.319	56.4
Dick+Cyat+Plag[4]	26	1265 - 1322	1334	230	17.2%	0.241	0.243	0.167	0.349	59.0
Dick+Cyat+Loxs[4]	26	1265 - 1322	1334	229	17.2%	0.239	0.258	0.185	0.318	55.7
Dick+Cyat+Meta[4]	26	1265 - 1322	1334	236	17.7%	0.248	0.266	0.176	0.310	55.8
Dick+Cyat+Plag+Loxs[4]	27	1265 - 1322	1334	255	19.1%	0.244	0.255	0.167	0.334	57.8
Tree Ferns[5]	28	1206 - 1322	1334	291	21.8%	0.244	0.264	0.165	0.328	57.2
Tree Ferns+*Azolla*	29	1206 - 1322	1334	343	25.7%	0.237	0.255	0.165	0.343	58.0

Table 3.1 Summary of Data from *rbcL* Gene: N°. seq. = number of sequences in the specified group; Length range = range of sequence lengths in the specified group; Aligned length = total length of the alignment; Single bp changes = the number of sites varying among the specified group; Percent variability = calculated as single bp changes / aligned length; Groups: Dicksoniaceae[1] = *Calochlaena*, *Cibotium*, *Culcita*, *Dicksonia*; Cyatheaceae[2] = *Alsophila*, *Sphaeropteris*; Dicksoniaceae + Cyatheaceae[3] includes *Lophosoria* and *Hymenophyllopsis*; [4] abbreviations Dick = Dicksoniaceae, Cyat = Cyatheaceae, Plag = Plagiogyriaceae, Loxs = Loxsomataceae, Meta = Metaxyaceae; Tree Ferns[5] includes all species except *Azolla caroliniana*. Base composition refers to variable sites only.

3.1.3 Usefulness Of *rbcL* Data For Tree Building

The usefulness of the data for tree-building was first investigated by split decomposition (Figure 3.1). This analysis used 1197 sites for which characters were unambiguous in all taxa (i.e. missing and ambiguous characters were excluded). Patterns in the data were found to be largely tree-like for ingroup taxa and therefore suitable for further investigation. However, the splits graphs provided a poor representation of ingroup relationships when outgroup sequences were also included in the analysis. The splits graph of these data showed long branch lengths leading to the outgroup taxa *Metaxya*, *Loxsoma*, *Plagiogyria* and particularly *Azolla*. Inclusion of

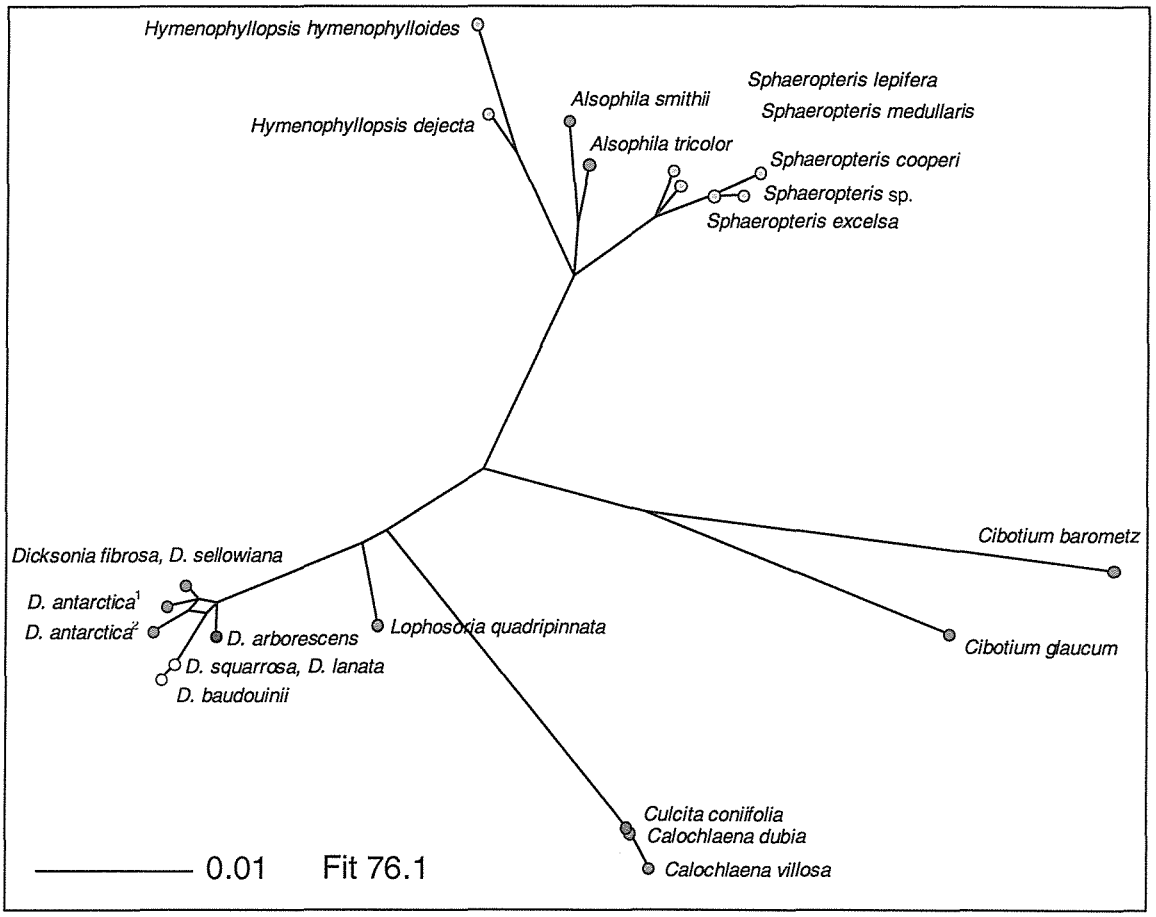

Figure 3.1 *rbcL* Splits Graph for Tree Fern Clade: includes the families Dicksoniaceae, Cyatheaceae, Lophosoriaceae and Hymenophyllopsidaceae. Colours indicate genera and in *Dicksonia*, spore type: smooth spore – red, tuberculate spore – yellow, intermediate spore – blue. *Dicksonia antarctica*[1] refers to sequences from Hasebe *et al.* 1994 and this study, while *Dicksonia antarctica*[2] is from Wolf *et al.* 1994. Note the long branches of the two *Cibotium* species. Least squares edge fitting is on.

these outgroup taxa in the reconstructed splits graph caused the collapse of the internal structure of the graph resulting in a 'star'.

In studying the ingroup under split decomposition the splits graph indicated that *Dicksonia* was most similar to *Lophosoria*, *Culcita* and *Calochlaena*. *Culcita* and *Calochlaena* were closely related genera. One point of interest that will be further discussed was a very short pendant branch leading to *Lophosoria*. In contrast *Cibotium* was highly divergent from the Cyatheaceae genera *Alsophila* and *Sphaeropteris* and the other Dicksoniaceae genera. The two species of *Cibotium* (one from Hawaii, the other Asia) were themselves separated by very long edges, with much more variation observed between them than for any other genus. *Hymenophyllopsis* was nested within the Cyatheaceae, producing a trichotomy with *Alsophila* and *Sphaeropteris*. Within *Sphaeropteris*, *S. lepifera* and *S. medullaris* were separate from the other species. Given the most likely root placement (see Figure 3.2) *S. excelsa* and *S. cooperi* were more derived.

3.1.4 Choosing A Substitution Model For Tree Building

For subsequent tree-building nested models of sequence evolution were evaluated for their fit to the data. Tests of fit were made on a Neighbour Joining tree assuming "p" (Hamming) distances. Table 3.2 shows the results of likelihood ratio tests identifying the most appropriate models. These were found to be the HKY+I+G, Fel84+I+G and GTR+I+G models. In evaluating parameters, empirical base compositions were assumed.

Table 3.3 shows the results of molecular clock tests made on ingroup taxa by comparing the log likelihood scores of the best trees constrained and unconstrained for rate variation along branches. These indicate some variation in rates of sequence evolution has occurred across the tree.

Tree Ferns *rbcL* (all sequences)									
Model	HKY	ΔW	χ2	Fel84	ΔW	χ2	GTR	ΔW	χ2
+G+I	4524.397			4523.721			4514.869		
+I	4639.636	230.478	3.841	4638.343	229.245	3.841	4621.209	212.681	3.841
-	4662.361	45.449	3.841	4661.011	45.337	3.841	4643.495	44.570	3.841
K2P	4670.591	16.461	9.488	4670.591	19.159	9.488	4670.591	54.193	9.488
JC69	4860.622	380.062	3.841	4860.622	380.062	3.841	4860.622	380.062	3.841
Tree Ferns (ingroup only)									
Model	HKY	ΔW	χ2	Fel84	ΔW	χ2	GTR	ΔW	χ2
+G+I	3202.953			3202.757			3201.354		
+I	3225.778	45.650	3.841	3225.509	45.505	3.841	3233.515	64.320	3.841
-	3230.104	8.652	3.841	3229.831	8.642	3.841	3227.794	-11.441	3.841
K2P	3233.148	6.087	9.488	3233.148	6.634	9.488	3233.148	10.707	9.488
JC69	3283.401	100.507	3.841	3283.401	100.507	3.841	3283.401	100.507	3.841

Table 3.2 Likelihood Ratio Test for *rbcL* Data: Three series of models were evaluated, HKY, Fel84 and GTR. ΔW values were compared with χ2 values to test for significance. In all cases, models incorporating an estimate of invariable sites and a gamma distribution (+G+I) were significantly better.

		Tree Score			
Tree	Outgroup	No Clock	Clock	ΔW	χ2
QP-ML	*Cibotium*	3206.745	3237.408	61.327	35.172
QP-ML	*Cibotium+Cyatheceae*	3206.745	3239.317	65.145	35.172
QP-ML	*Cyatheaceae*	3206.745	3276.162	138.836	35.172
NJ	*Cibotium*	3201.140	3234.182	66.084	35.172
NJ	*Cibotium+Cyatheceae*	3201.140	3236.891	71.500	35.172
NJ	*Cyatheaceae*	3201.140	3236.891	71.500	35.172

Table 3.3 Molecular Clock Test for *rbcL* Data: Two trees were evaluated for their fit to a molecular clock, a Quartet Puzzling Maximum Likelihood tree (QP-ML) and a Neighbour Joining tree (NJ). ΔW values were calculated from comparison of tree scores rooted at 3 different positions, and compared with χ2 values to test for significance. All trees failed indicating the data does not conform to a molecular clock.

3.1.5 Outgroup Selection

A Likelihood Ratio Test was performed to determine which, if any, of the outgroup sequences available were appropriate for use in tree-building. This was done by estimating parameters for the GTR+I+G model on a NJ tree built from "p" (Hamming) distances, with the ingroup and one or several outgroup taxa included. These parameter settings were then saved separately. A QP tree of the ingroup taxa was then generated under the same model and the tree score recorded. A new tree score was then generated under the parameters for each outgroup or set of outgroups

and this was compared with the score from the ingroup tree. Results are presented in Table 3.4.

	Ingroup Tree Scores			
Outgroup(s)	**Ingp+Outgp**	**Ingroup**	**ΔW**	**χ2**
Azolla	3206.76803	3201.14392	11.248	3.841
Plagiogyria	3202.19315	3201.14392	2.098	3.841
Metaxya	3202.24305	3201.14392	2.198	3.841
Loxsoma	3202.56609	3201.14392	2.844	3.841
Lox,Pla	3203.53897	3201.14392	4.790	3.841
Met,Lox,Pla	3204.72973	3201.14392	7.172	3.841
Azo,Met,Lox,Pla	3209.63736	3201.14392	16.987	3.841

Table 3.4 Likelihood Ratio Test for Outgroups: Outgroup taxa were examined using a likelihood ratio test. The score of a QP tree built from ingroup taxa was compared with the score for the same tree under GTR+I+G parameters estimated with outgroup taxa included. "Ingp+Outgp" indicates tree scores under the parameters estimated with the specified outgroup taxa included. "Ingroup" indicates the tree score under ingroup parameters. *Metaxya*, *Loxsoma* and *Plagiogyria* individually passed the test indicating one of them might be appropriate to use as an outgroup.

Azolla failed the test, indicating it is not an appropriate choice as an outgroup as its substitution properties differ significantly from those of the ingroup. *Metaxya, Loxsoma* and *Plagiogyria* all passed the test individually, but when more than one of these was included they failed the test. To identify possible root placement, a tree was built including the ingroup taxa with each of these outgroups. *Loxsoma* and *Plagiogyria* both rooted in the same place, on the branch leading to *Dicksonia*, *Lophosoria*, *Culcita* and *Calochlaena*. *Metaxya* rooted further along this branch, resulting in a polytomy. As it altered the relationships of the ingroup taxa *Metaxya* was clearly not a good choice for an outgroup. Midpoint rooting of the ingroup taxa was also performed for comparison. This resulted in placement of the root on the *Cibotium* branch.

3.1.6 Evolutionary Trees

The GTR+I+G model with parameter settings estimated in section 3.1.4 was used under Quartet Puzzling (QP) and NJ tree selection criteria to reconstruct phylogenies for the *rbcL* data. The Maximum Parsimony criterion was also used under QP for

comparison. *Plagiogyria* was chosen for use as an outgroup based on results from section 3.1.5.

The resulting tree was very similar in shape to the splits graph (Figure 3.1). The position of internal groups (i.e. the Cyatheaceae + *Hymenophyllopsis*, *Dicksonia* + *Lophosoria* + *Culcita*/*Calochlaena*, and *Cibotium*) remained identical. *Plagiogyria* rooted the tree on the *Dicksonia* + *Lophosoria* + *Culcita*/*Calochlaena* branch. A Neighbour Joining tree using the GTR model with the proportion of invariable sites and gamma distribution previously estimated by maximum likelihood was also similar in tree topology. A Quartet Puzzling tree using Maximum Parsimony as the optimality criterion also resulted in a tree almost identical to the previous trees. Bootstrap and Puzzle values from all three trees are shown in Figure 3.2. In most cases there is good agreement between all three trees. The position of *Lophosoria* as closer to *Dicksonia*, which is only unsupported in the NJ tree, is the most incongruent split. The support values for root placements on the three major branches, from 1) the ML tree, 2) the MP tree and, 3) a NJ bootstrap tree are as follows: *Calochlaena* + *Culcita* + *Dicksonia* + *Lophosoria*: 74, 94, 51; *Cibotium* 25, 6, 49; *Alsophila* + *Hymenophyllopsis* + *Sphaeropteris*: <5, <5, <5.

3.2 Discussion

The splits graph reconstructed with outgroups (*Azolla*, *Metaxya*, *Loxsoma* and *Plagiogyria*) resulted in a star structure due to the collapse of internal edges. This problem has been observed previously (Lockhart *et al.* 2001) and is caused by including taxa with very long branches in a split decomposition analysis. Exclusion of these taxa produced a splits graph with almost no reticulation, indicating that the ingroup data was tree-like and suitable for phylogeny reconstruction.

Results from likelihood ratio tests showed complex models of sequence evolution were a better fit for the data than simpler models (Table 3.2). This may be expected due to the nature of the *rbcL* gene. Simple models such as the Jukes-Cantor (1969) and Kimura 2-parameter (1980) models assume equal base frequencies. Base

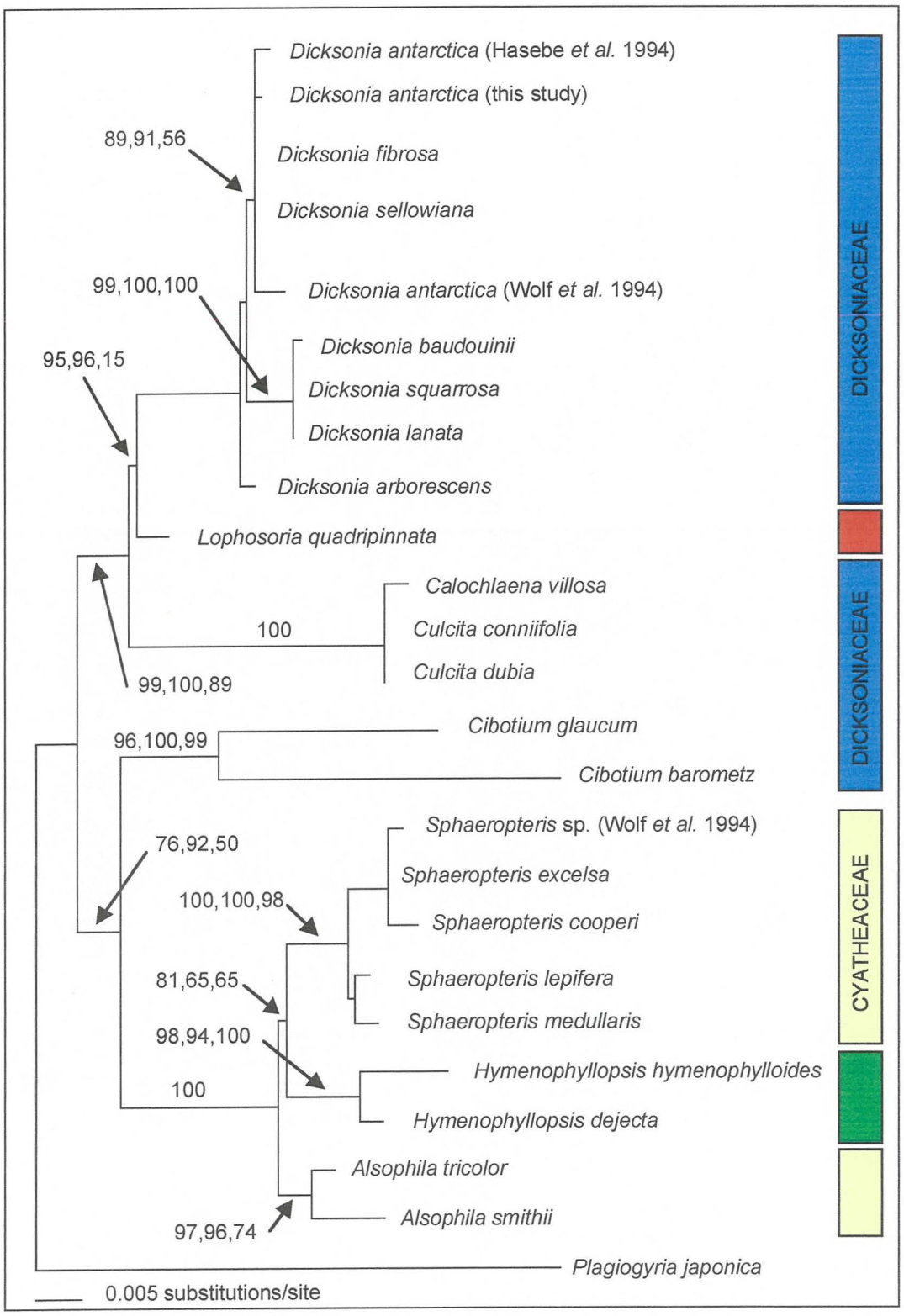

Figure 3.2 Quartet Puzzling Tree for Tree Fern *rbcL* Sequences: The optimality criterion used is Maximum Likelihood with the GTR+I+G model. The three numbers on the splits indicate: 1) Puzzle values from the ML tree, 2) Puzzle values from an MP tree and 3) Bootstrap values from an NJ tree. For equal values only a single number is shown. In right hand bars red indicates Lophosoriaceae and green indicates Hymenophyllopsidaceae.

compositions of the variable sites for the *rbcL* sequences used in this study are clearly unequal (Table 3.1), and thus the more complex models using unequal base frequencies would be expected to better fit the data. Similarly, more complex models assuming a proportion of invariable sites and a gamma distribution of variable sites were found to better fit the data. This was also not surprising given that *rbcL* is a coding sequence. First and second codon positions will be expected to be invariable or have low variability, while third codon positions will be quite variable.

Molecular clock analyses performed on the ingroup taxa (excluding *Metaxya*, *Loxsoma* and *Plagiogyria*) showed that the data did not conform to a molecular clock model. This was also indicated by the differing branch lengths evident in both the splits graph and PAUP* trees. In particular, the *Lophosoria* branch was very short, while the *Cibotium* and *Culcita/Calochlaena* branches were disproportionately long.

3.2.1 Rooting the Tree Fern Clade

As mentioned in section 1.1, the existence of a 'tree fern clade' encompassing several related families has now been recognised, although the relationships between members has remained uncertain. One aspect of uncertainty concerns producing a rooted phylogeny for the group. The outgroups studied here, *Metaxya*, *Loxsoma*, and *Plagiogyria*, all show very long branches relative to the ingroup taxa, and are possibly too distant from the group consisting of the Cyatheaceae, Dicksoniaceae, Hymenophyllopsidaceae and Lophosoriaceae to provide a strong indication of the root position. There is also some support for a sister relationship between *Loxsoma* and *Plagiogyria*, although the branch lengths between them are long. Given the extent of divergence, multiple substitutions have probably resulted in loss of too much phylogenetic signal to resolve the relationship of these taxa to the Dicksoniaceae and Cyatheaceae.

The problem with using outgroups to root the tree is evident in both split decomposition and standard tree building analyses. Likelihood Ratio Tests suggested of those sequences available, some were clearly evolving under different models of evolution than the ingroup sequences. For this reason the inferences of root placement indicated (Figure 3.2) are tentative. The tree was rooted using *Plagiogyria* (arbitrarily

chosen over *Loxsoma*) as an outgroup, as *Metaxya* and *Azolla* were shown to be inappropriate choices (see 3.1.5). As the *rbcL* sequences were shown to be evolving in a non-clock-like fashion midpoint rooting would also have been a poor choice. The issue of root placement may be better studied with a more conserved marker such as *18S*.

3.2.2 Major Features of the Tree Fern Clade

Within the internal group there are three major lineages. The first of these consists of the genera *Dicksonia*, *Culcita*, *Calochlaena* and *Lophosoria*. The second contains the genera *Alsophila*, *Sphaeropteris*, and *Hymenophyllopsis*. The final lineage is that of *Cibotium*. In some trees (e.g. Figure 3.2) there is evidence that *Cibotium* is more closely related to the Cyatheaceae, which would indicate that the Dicksoniaceae, as currently recognised, is paraphyletic, and that the Cyatheaceae have arisen from within the Dicksoniaceae, as traditionally suggested from morphological evidence. However, two almost equally supported root placements remain possible. These root the tree on the *Cibotium* edge, and the other partitions *Cibotium* with the Dicksoniaceae.

3.2.3 Lineages

Within the *Dicksonia* + *Lophosoria* + (*Calochlaena* + *Culcita*) lineage relationships appear well resolved. In the reconstructed graph (Figure 3.2) *Culcita* and *Calochlaena* form a close group, the latter having only recently been recognised as a separate genus. The lack of variation between them may indicate that generic separation is unwarranted. *Dicksonia* appears more closely related to *Lophosoria* than any of the other genera of the Dicksoniaceae, but again branch lengths are relatively long indicating the divergence is quite old. Fossil evidence suggests that these three groups may have diverged early in the Tertiary (65 – 2 million years ago). Molecular estimates of date may not be possible due to the absence of a molecular clock within the tree fern clade, at least for *rbcL*.

In the second lineage, consisting of the two *Cibotium* species, the branch lengths between the two species are exceedingly long (e.g. Figure 3.1). This perhaps indicates

a long separation between the Asian and American species. It has been suggested that speciation within *Cibotium* took place around the Eocene (54.9 – 38 mya, Tidwell & Nishida, 1993), although the branch lengths shown here indicate that may be a substantial underestimate. By comparison the species of all the other genera appear to be very recent, showing a general pattern of old lineages with relatively recent speciation. The root placement shown in Figure 3.2 indicates that *Cibotium* may be a sister group to the Cyatheaceae.

The Cyatheaceae lineage provides perhaps the most interesting finding, with the relationship of *Hymenophyllopsis* to the Cyatheaceae genera *Alsophila* and *Sphaeropteris*. In Figure 3.1 the three genera appear to form a trichotomy, while the QP tree (Figure 3.2) places *Hymenophyllopsis* slightly closer to *Sphaeropteris*. In any case, *Hymenophyllopsis* certainly appears to be nested within the Cyatheaceae. In Wolf *et al.* (1999) this genus was shown to be sister to *Cyathea*. However, this placement was based on a single sequence (reported as *Cyathea*, but actually *Sphaeropteris*). Additional sequences added in this study, representing both *Alsophila* and *Sphaeropteris*, have resolved this further to show *Hymenophyllopsis* as an internal branch of the Cyatheaceae. This finding will be discussed further in section 5.2.2.

3.2.4 Familial Relationships

Without a doubt the familial relationships within the 'tree fern clade' need revision. In their present state the families are paraphyletic and are unrepresentative of evolutionary relationships. However, two genera remain unsequenced within the Dicksoniaceae, *Cystodium* and *Thyrsopteris*. Given the differences between morphological and molecular studies to date it is difficult to suggest where these two genera would fit into a phylogeny. It has been suggested that they are only distantly related to the other genera within the Dicksoniaceae (Tryon & Tryon 1982). The spores of *Cystodium* have been noted to resemble those of *Metaxya* (Tryon & Lugardon 1991), and the genus also lacks an arborescent habit, suggesting a possible placement outside the Dicksoniaceae. Similarly, the spores of *Thyrsopteris* also suggest a more distant relationship to the Dicksoniaceae (Gastony 1981). *Thyrsopteris* has a chromosome count of n = 76 - 78, and *Cystodium* an unconfirmed count of n = 56, both of which differ considerably from the other Dicksoniaceae and Cyatheaceae,

which are all in the range of n = 65 - 69. Until sequencing work has been performed on these taxa such a revision would be premature.

The families Hymenophyllopsidaceae and Lophosoriaceae should clearly be abandoned, and the single genus of each moved into the Cyatheaceae and Dicksoniaceae respectively. If these two families are retained, placing *Cibotium* in the Cyatheaceae would seem appropriate, as this appears to be a natural split. However, this relationship of *Cibotium* with the Cyatheaceae goes against some of the traditional tenets of familial division. The two tree fern families, Dicksoniaceae and Cyatheaceae are traditionally separated on the basis of soral position on the pinnae, with Dicksoniaceae possessing marginal sori, while sori in the Cyatheaceae are borne away from the margins. *Cibotium* possesses marginal sori that have traditionally placed it in the Dicksoniaceae. Similarly, in *Lophosoria* the sori are borne on leaf veins, like the Cyatheaceae. However, the *rbcL* trees here place it clearly closer to the Dicksoniaceae. A single family encompassing all the Dicksoniaceae and Cyatheaceae, as well as the Hymenophyllopsidaceae and Lophosoriaceae may be in order. *Cystodium* and *Thyrsopteris* may well fall outside this group.

3.2.5 The Arborescent Habit

Stevenson and Loconte (1996), from a tree based on morphological characters, proposed that the tree habit had arisen independently 3 times within the tree fern clade. This was due to the placement of the Loxsomataceae as a sister group to *Cyathea-Lophosoria*, and the lack of tree habit in *Culcita*, *Calochlaena* and *Cystodium*. The *rbcL* data in this thesis suggest that *Loxsoma* falls outside the Dicksoniaceae and Cyatheaceae with *Metaxya* and *Plagiogyria*. From this it is most parsimonious to assume a single origin of the arborescent tree habit among these taxa, with subsequent reduction, or loss in some lineages. *Hymenophyllopsis*, for example, which seems to be firmly nested within the Cyatheaceae, appears to have become very reduced in size, but still retains some semblance of an upright stem. This appears to be a relatively common phenomenon. Several species within the Cyatheaceae and Dicksoniaceae have also lost the arborescent tree habit, *Alsophila colensoi* of New Zealand for example, and many species which are normally erect may have prostrate form, e.g. *Dicksonia lanata*, *A. tricolor*. It could be supposed that a gene or genes

enabling the arborescent habit arose once, and subsequent mutations or control of gene expression can result in reduction or loss of this habit. The genetic basis for this requires further investigation.

4.1 Results

Results from analysis of the genus *Dicksonia* using DNA sequencing and AFLP techniques are reported here. Sequencing of molecular markers was used to study relationships among members of the genus, while AFLP was used to examine the population structure of the New Zealand species *D. lanata*.

4.1.1 DNA Extractions

The CTAB procedure utilised in this study worked best on fresh, undried material, especially that which was young and soft. Samples dried in silica gel which were taken from similar material also worked well. Silica gel dried samples that were taken from older, harder tissues often failed to produce precipitated DNA that could be isolated without centrifugation. Spinning such DNA usually resulted in a dirty, brown pellet. This DNA was of sufficient quality for PCR, but not for AFLP. Samples of fertile fronds generally resulted in large tissue fragments in the top layer after the chloroform step. This was due to the sori being tough and difficult to crush by grinding with a glass rod. Most of this unbroken tissue could be avoided by careful pipetting, although some tissue fragments occasionally remained, and became tangled in the DNA at the precipitation step.

DNA was successfully extracted from all available *Dicksonia* species, with the exception of a few *D. lanata* samples. These were from the Herekino and Whangarei populations of the Northern form and the Rangiwahia, Mokai, Mangaweka and Kaweka populations of the Southern form. These samples were of older, dried tissue and failed to yield DNA from repeated extraction attempts. In most cases other nearby populations were successfully extracted. DNA was extracted from multiple populations of *D. fibrosa* and *D. squarrosa* (Voucher specimens and details in Appendix 2.1).

DNA was also obtained from the foreign *Dicksonia* species *D. antarctica*, *D. arborescens*, *D. baudouinii*, *D. berteriana* and *D. sellowiana*.

Samples from *Lophosoria quadripinnata* and *Cibotium glaucum* were also obtained for use as outgroups to the *Dicksonia* species. *Lophosoria* yielded a large amount of clean DNA. However, extraction from *Cibotium* was less successful, resulting in a small amount of dirty, brown DNA.

4.1.2 PCR and Sequencing

rps4

The *rps4* region is a chloroplast gene coding for a ribosomal small sub-unit protein which has been utilised successfully for phylogenetic analysis (e.g. Nadot *et al.* 1994). PCR amplification of the *rps4* region in *Dicksonia* was unsuccessful. Weak amplification was observed in both *D. lanata* and *D. squarrosa* using rps4F and trnS primers. Second round amplifications using these products with internal primers, rps2 and trnS, produced multiple bands when run on an agarose gel. Further attempts to amplify this locus were abandoned after successful amplification of the *trnL-trnF* spacer, an alternative chloroplast marker that is also considered to be fast evolving (Gielly & Taberlet 1995).

trnL-trnF spacer

The *trnL-trnF* spacer is a short, non-coding spacer region lying between two tRNA genes, which can be phylogenetically informative (Taberlet *et al.* 1991). A first attempt at amplification of the entire spacer region using the TabE and TabF (see Appendix 2.3) primers yielded bright bands from both *D. lanata* and *D. fibrosa* of approximately 400 bp in size. These products were cleaned and sequenced. No internal primers were required due to the short length of the amplified region. The DNA sequences showed several single base pair differences and an insertion/deletion (indel) between them. Successive attempts to PCR other species (including *D. antarctica*, *D. baudouinii*, *D. berteriana*, *D. sellowiana*, *D. squarrosa*, *D. youngiae*

and *Lophosoria quadripinnata*) using these primers were all successful. PCR of *Cibotium glaucum* was unsuccessful due to the poor quality of the extracted DNA.

trnL intron

The *trnL* intron is found in the *trnL* gene 5' of the *trnL-trnF* spacer. As a non-coding region it is also fast evolving (Gielly & Taberlet 1995). Attempts to amplify this region with PCR, using primer combinations TabC with TabD, and TabC with TabF produced multiple bands on a 1% agarose gel. Second round amplification of the TabC-TabF products using TabC and TabD also failed to yield single products. Further attempts to amplify this region were abandoned, as it was found that there was sufficient signal within the *trnL-trnF* spacer region alone to distinguish most species.

rbcL

The *rbcL* gene has been widely used for phylogenetic studies, in a range of taxa including ferns (e.g. Hasebe *et al.* 1995). The large number of sequences available for various tree ferns available from GenBank prompted attempts here to amplify this region in NZ and other *Dicksonia* species. Unpublished *rbcL* sequences of *D. lanata* and *D. squarrosa* previously obtained by L. R. Perrie were also available. Sequences from these two species were identical across their length (>1.3 kb).

Five primers were used to obtain double stranded sequence for additional *Dicksonia* species (aF, cR, 422F, 961F and 579R: Appendix 2.3). Sequencing of the *rbcL* region was successful using all initial primers except for 579R. This primer appeared to differ from its target sequence in *Dicksonia* at three sites, one of which appeared to be critical to primer binding. This was suggested from the observation that *Sphaeropteris excelsa* and *S. medullaris* matched the primer sequence at this base and differed in two others, but were readily sequenced using this primer. A new primer to replace 579R was designed so as to exactly match sequences in *Dicksonia* and *Alsophila* (which were identical over the primer region). This primer was named 579Rdic, and was found to be successful in all species sequenced. The complete sequenced open reading frame in *Dicksonia* was 1320 bp in length.

ITS

The *ITS* region is a fast evolving non-coding nuclear region present in multiple copies with well documented phylogenetic usefulness (e.g. Baldwin *et al.* 1995). PCR of this marker using primers ITS4 with ITS5, and ITS2 with ITS5, resulted in multiple bands from repeated attempts. Further PCR of this marker was abandoned in favour of *18S*.

18S

The *18S* region is found in the nuclear genome and codes for a rRNA. It is highly conserved and most useful for studying higher level phylogenetic relationships (e.g. Soltis *et al.* 1999). PCR amplification of this region was accomplished using the primers 18sF and 18sR. Four *Dicksonia* species were characterised: *D. antarctica*, *D. arborescens*, *D. fibrosa* and *D. lanata*. Additionally, *Lophosoria quadripinnata* was also included. PCR products were approximately 1.8 kb in length on an agarose gel. DNA sequencing was performed using a total of four primers, the external 18sF and 18sR, and internal 18sF2 and 18sR2. This gave double stranded sequence for only the middle c. 600 bp of the 18sF and 18sR fragment. Either side of this region only single stranded sequence was determined, but was clearly readable. *18S* sequences obtained in this study showed very low variability, and consequently complete sequencing of both strands was not considered worthwhile.

4.1.3 DNA Sequence Alignments

Positional homology among DNA sequences was inferred using ClustalX as described in section 2.5.2. Complete alignments for *rbcL* may be found in Appendix 3.1 and alignments for *trnL-trnF* and *18S* in Appendix 4.1.

trnL-trnF

The alignment of DNA sequences from the *trnL-trnF* spacer for *Dicksonia* was 443 bp in length. The shortest individual sequence was from *D. lanata* at 390 bp. The longest sequence was from *D. squarrosa* at 400 bp. *Lophosoria quadripinnata* was also included in this alignment, with a sequence length of 412 bp. There was some

sequence variation between the *Dicksonia* species, 12 single base pair changes and 4 indels (see Table 4.1).

rbcL

The *rbcL* sequences obtained were aligned with all other available tree fern clade sequences, as described in section 3.1. Only *Dicksonia lanata* and *D. squarrosa* were less than full length, at 1245 and 1230 bp, respectively. All others were 1320 bp. Within the genus *Dicksonia*, sequences showed 16 sites that varied. When the genus *Lophosoria* (the closest sequence, see 3.1.3) was included the number of sites that varied increased to 33. No indels were present among the sequences.

18S

The four *18S* sequences obtained from *Dicksonia* (Three from sequencing: *D. arborescens*, *D. fibrosa*, *D. lanata*; one from GenBank: *D. antarctica*) were aligned with three from the Cyatheaceae (*Alsophila smithii*, *A. tricolor*, *Sphaeropteris medullaris*), a *Lophosoria quadripinnata* sequence, and a sequence from *Adiantum raddianum*. The full length of the *18S* region is 1811 bp in most of the GenBank fern taxa. None of the new sequences obtained were full length, ranging from 1691 bp in *Sphaeropteris medullaris* to 1742 bp in *Alsophila tricolor*.

An alignment of available fern *18S* sequences from GenBank showed a consistent 5 bp discrepancy (CATGN) in many of the sequences, at 1187 - 1191 bp. This was also observed in the *Dicksonia antarctica* sequence from GenBank. This discrepancy was investigated by sequencing *D. antarctica* using the primers 18sR and 18sF2. These primers flanked the area in question. The sequence obtained was then used to correct the GenBank *D. antarctica* sequence. This additional sequencing also identified a further 2 bp error in the GenBank *D. antarctica* sequence.

Using the corrected *Dicksonia antarctica* sequence in the final alignment reduced the observed variation within *Dicksonia* to two substitutions. A single bp change separated *Dicksonia* from *Lophosoria*. Approximately 7 bp changes separated

Marker + Taxa	No. Seq.	Length Range	Aligned Length	Single bp Changes	Indels	Percent Variability	Base Composition A	C	G	T	% A-T
trnL-F (chloroplast)											
Dicksonia	10	390 - 400	443	12	4	3.61%	0.119	0.286	0.310	0.286	40.48
Dicksonia+Lophosoria	11	390 - 412	443	26	8	7.67%	0.159	0.216	0.349	0.276	43.51
rbcL (chloroplast)											
Dicksonia	10	1230 - 1320	1334	16	0	1.20%	0.138	0.288	0.256	0.319	45.63
Dicksonia+Lophosoria	11	1230 - 1322	1334	33	0	2.47%	0.096	0.375	0.190	0.339	43.53
18S (nuclear)											
Dicksonia	4	1708 - 1724	1811	2	0	0.06%	0.000	0.750	0.000	0.250	25.00
Dicksonia+Lophosoria	5	1708 - 1727	1811	3	0	0.11%	0.267	0.533	0.067	0.133	40.00
Dicksonia+Cyatheaceae1	7	1691 - 1742	1811	12	0	0.66%	0.224	0.367	0.102	0.306	53.06
Dicksonia+Adiantum	5	1708 - 1811	1811	9	0	0.50%	0.077	0.523	0.062	0.338	41.54
All Sequences2	9	1691 - 1811	1811	19	0	1.05%	0.146	0.414	0.106	0.333	47.98

Table 4.1 Summary of DNA Sequence Marker Data for the Genus *Dicksonia*: N°. Seq. = number of sequences in specified group; Length Range = range of sequence lengths within specified group; Aligned Length = aligned sequence length including gaps; Single bp changes = number of sites with single nucleotide substitutions among specified group; Indels = number of insertions or deletions present among specified group; Percent Variability = estimate of sequence variability within specified group; calculated as (Single bp changes + Indels) / Aligned length. Cyatheaceae[1] = *Alsophila*, *Sphaeropteris*. All Sequences[2] = *Dicksonia*, *Lophosoria*, *Alsophila*, *Sphaeropteris*, *Adiantum*. Complete lists of taxa sequenced for each marker may be found in the alignments in Appendices 3.1 and 4.1.

Dicksonia from the Cyatheaceae sequences, and 9 bp between *Dicksonia* and *Adiantum*. See 4.2 for further discussion.

4.1.4 Misidentified Taxa

Sequence analysis suggested potential problems with some materials sourced for the present study. Alignment of *trnL-trnF* sequences from *Dicksonia* showed two species had been misidentified. A sample labelled as *Dicksonia berteriana* from Chile returned sequences identical to *D. sellowiana*, for both the *trnL-trnF* and *rbcL* markers. Spore morphology verified it as *D. sellowiana*. Another samples, labelled as *Dicksonia youngiae* from the New Zealand garden industry similarly proved to be misidentified. A *trnL-trnF* sequence from this sample was identical to *D. antarctica*, indicating that what is sold in New Zealand garden centres as *D. youngiae* may in fact be *D. antarctica*. In both cases, the samples were supposedly from members of the tuberculate spore group, but sequencing and spore morphology showed them to be from smooth-spored species.

4.1.5 Usefulness Of Sequence Data For Tree Building

The usefulness of the collected sequence data for tree building was first investigated using split decomposition.

trnL-trnF

First, the *trnL-trnF* data from *Dicksonia* and *Lophosoria* was examined using SplitsTree 3.1. Of the full 443 bp alignment, 384 characters were used in the analysis (with missing and ambiguous characters excluded). The resulting splits graph (Figure 4.1) was largely tree-like, except for reticulation caused by a character placing *Dicksonia lanata* and *D. squarrosa* with the outgroup, *Lophosoria*. The fit statistic was very high, at 99.7. In this splits graph *Dicksonia* formed three distinct lineages, of approximately equal edge length, with a long branch leading to *Lophosoria*. The first lineage consisted of *D. fibrosa*, *D. antarctica* and *D. sellowiana*, with a single variable site between them. The second lineage had a sole member, *D. arborescens*. The third lineage consisted of *D. lanata*, *D. squarrosa* and *D. baudouinii*. The most

variation was observed within this group. With all 443 characters included the topology of the graph was identical.

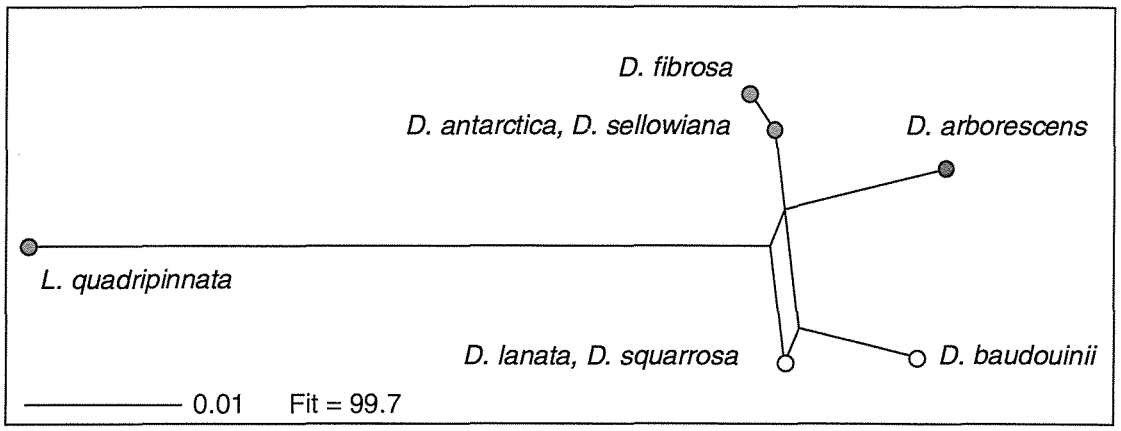

Figure 4.1 *trnL-trnF* Splits Graph for *Dicksonia*: Including seven species of *Dicksonia* falling into three groups: *D. fibrosa*, *D. antarctica* and *D. sellowiana* with smooth spores (red); *D. lanata*, *D. squarrosa* and *D. baudouinii* with tuberculate spores (yellow); and *D. arborescens* with intermediate smooth/tuberculate spores (blue). *Lophosoria quadripinnata* is included as an outgroup (green).

18S

Data from the nuclear *18S* marker was also analysed using split decomposition. Excluding missing and ambiguous sites left 1671 sites for analysis. The resulting splits graph (Figure 4.2) was also tree-like except for some slight reticulation caused by *Lophosoria*. While the level of sequence variation was very low for this marker, the general pattern of the graph matched that observed in the *rbcL* graphs from Chapter 3. The relationships among the *Dicksonia* species are not resolved by analysis of *18S* sequences due to lack of phylogenetic signal. However, in these data there is sufficient signal to separate the Cyatheaceae genera *Alsophila* and *Sphaeropteris* from *Dicksonia*.

rbcL

The *rbcL* sequences for *Dicksonia* had already been determined to be tree-like and suitable for tree building as part of a larger data set in Chapter 3.

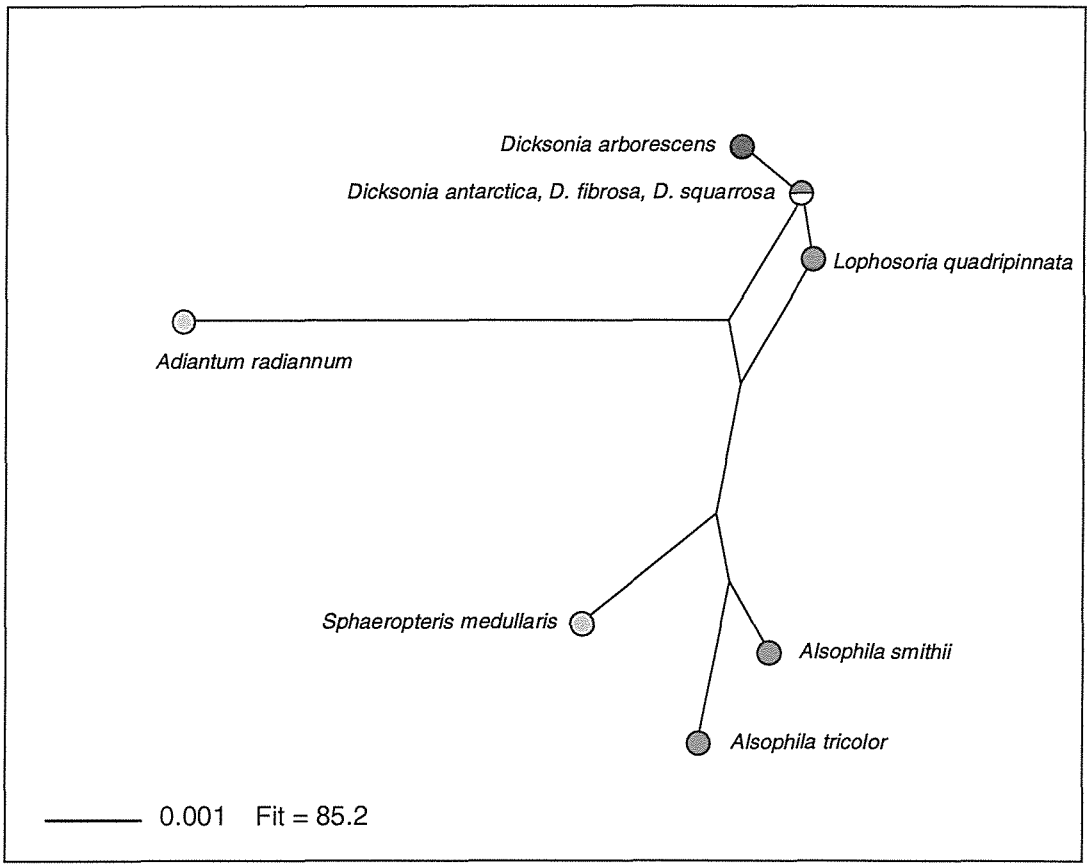

Figure 4.2 *18S* Splits Graph: Includes four *Dicksonia* species, *D. arborescens* of intermediate spore type (blue), *D. antarctica* and *D. fibrosa* of smooth spore type (red) and *D. lanata* of tuberculate spore type (yellow). Note the three-way split between spore types observed in the *trnL-trnF* marker collapses under *18S*. Also included are *Lophosoria quadripinnata* (green), *Alsophila smithii*, *A. tricolor*, *Sphaeropteris medullaris* and *Adiantum raddianum* as an outgroup. Least squares edge fitting is on.

4.1.6 Choosing A Substitution Model For Tree Building

Having identified the sequence data as suitable for tree building, nested models of sequence evolution were evaluated for their fit to the data, as in Chapter 3. Tests of fit were made on a Neighbour Joining tree assuming "p" (Hamming) distances. Table 4.2 shows the results of likelihood ratio tests used to determine the most appropriate model for each DNA sequence marker. For the *trnL-trnF* spacer region the HKY, Fel84 and GTR models were found to be most appropriate, without assuming invariable sites or a gamma distribution of site variability. For the *18S* marker the HKY+I+G, Fel84+I+G and GTR+I+G models were found to be the best models. For *rbcL* the K2P and GTR models were identified as being most appropriate.

Dicksonia trnL-trnF

Model	HKY	ΔW	χ2	Fel84	ΔW	χ2	GTR	ΔW	χ2
+G+I	713.156			713.178			711.421		
+I	713.159	0.007	3.841	713.181	0.007	3.841	711.421	0.000	3.841
-	713.163	0.008	3.841	713.185	0.008	3.841	711.421	0.000	3.841
K2P	721.653	16.979	9.488	721.653	16.935	9.488	721.653	20.463	9.488
JC69	727.950	12.594	3.841	727.950	12.594	3.841	727.950	12.594	3.841

Dicksonia rbcL

Model	HKY	ΔW	χ2	Fel84	ΔW	χ2	GTR	ΔW	χ2
+G+I	1938.355			1938.252			1930.487		
+I	1939.986	3.263	3.841	1939.873	3.243	3.841	1931.797	2.620	3.841
-	1940.456	0.940	3.841	1940.342	0.938	3.841	1932.237	0.881	3.841
K2P	1943.959	7.006	9.488	1943.959	7.233	9.488	1943.959	23.444	9.488
JC69	1954.540	21.161	3.841	1954.540	21.161	3.841	1954.540	21.161	3.841

Dicksonia, Lophosoria,* Cyatheaceae *18S

Model	HKY	ΔW	χ2	Fel84	ΔW	χ2	GTR	ΔW	χ2
+G+I	2504.624			2504.066			2494.995		
+I	2511.380	13.513	3.841	2510.804	13.476	3.841	2501.700	13.410	3.841
-	2512.270	1.778	3.841	2511.692	1.776	3.841	2502.587	1.774	3.841
K2P	2517.786	11.032	9.488	2517.786	12.187	9.488	2517.786	30.398	9.488
JC69	2535.975	36.379	3.841	2535.975	36.379	3.841	2535.975	36.379	3.841

Table 4.2 Likelihood Ratio Test on Sequence Data: Three series of models were evaluated, HKY, Fel84, and GTR. ΔW values were compared with χ2 values to test for significance. For *trnL-trnF* the HKY, Fel84 and GTR models were most appropriate, for *18S* the HKY+I+G, Fel84+I+G and GTR+I+G models, and for *rbcL* the K2P and GTR models.

18S		Tree Score			
Tree	**Root**	**No Clock**	**Clock**	**ΔW**	**χ2**
QP-ML	*Adiantum*	2485.538	2500.082	29.089	14.067
QP-ML	Midpoint	2485.538	2504.798	38.520	14.067
QP-ML	Cyatheaceae	2485.538	2502.418	33.761	14.067
NJ	*Adiantum*	2485.580	2500.74	30.319	14.067
NJ	Midpoint	2485.580	2505.447	39.733	14.067
NJ	Cyatheaceae	2485.580	2503.057	34.952	14.067

Table 4.3 Molecular Clock Test for 18S: Two trees were evaluated for their fit to a molecular clock, a Quartet Puzzling Maximum Likelihood tree (QP-ML) and a Neighbour Joining tree (NJ). ΔW values were calculated from comparison of tree scores rooted in different positions, with or without a molecular clock enforced, and compared with χ2 values to test for significance. All trees failed indicating the data does not conform to a molecular clock.

Molecular clock tests were performed on the *18S* and *trnL-trnF* data. Tables 4.3 and 4.4 show the results of these tests, which were made by comparing the log likelihood

scores of the best trees constrained and unconstrained for rate variation along branches. For both markers there is no indication of variation in rates of sequence evolution across the trees.

Dicksonia *trnL-F*		Tree Score			
Tree	Root	No Clock	Clock	ΔW	χ2
QP-ML	*Lophosoria*	711.299	713.850	5.103	19.675
QP-ML	*D. arborescens*	711.299	723.817	25.037	19.675
QP-ML	*D. lanata* group	711.299	723.817	25.037	19.675
NJ	*Lophosoria*	711.299	714.098	5.599	19.675
NJ	*D. arborescens*	711.299	724.083	25.568	19.675
NJ	*D. lanata* group	711.299	724.083	25.568	19.675

Table 4.4 Molecular Clock Test for *trnL-trnF* in *Dicksonia*: Two trees were evaluated for their fit to a molecular clock, a Quartet Puzzling Maximum Likelihood tree (QP-ML) and a Neighbour Joining tree (NJ). ΔW values were calculated from comparison of tree scores rooted in different positions, with or without a molecular clock enforced, and compared with χ2 values to test for significance. Trees rooted with *Lophosoria* passed, indicating the marker is evolving in a clock-like fashion.

4.1.7 Evolutionary Trees

trnL-trnF

The GTR model with parameters estimated in 4.1.6 was used under Quartet Puzzling (QP) and Neighbour Joining (NJ) tree selection criteria to reconstruct phylogenies for *Dicksonia* with the *trnL-trnF* marker. Maximum Parsimony was also used under QP for comparison. *Lophosoria* was used as an outgroup.

In both the NJ and MP trees *D. arborescens* (intermediate spore type) was placed closer to the smooth spore group (*D. antarctica*, *D. fibrosa*, *D. sellowiana*) than the tuberculate spore group (*D. baudouinii*, *D. lanata*, *D. squarrosa*). In the ML tree, the three spore types formed a trichotomy.

rbcL

The *rbcL* data from *Dicksonia* and *Lophosoria* was also analysed using QP and NJ selection criteria with the GTR model. A Maximum Parsimony QP tree was again

constructed for comparison. These analyses used 1226 sites for which the data was unambiguous. Results are shown in Figure 4.4.

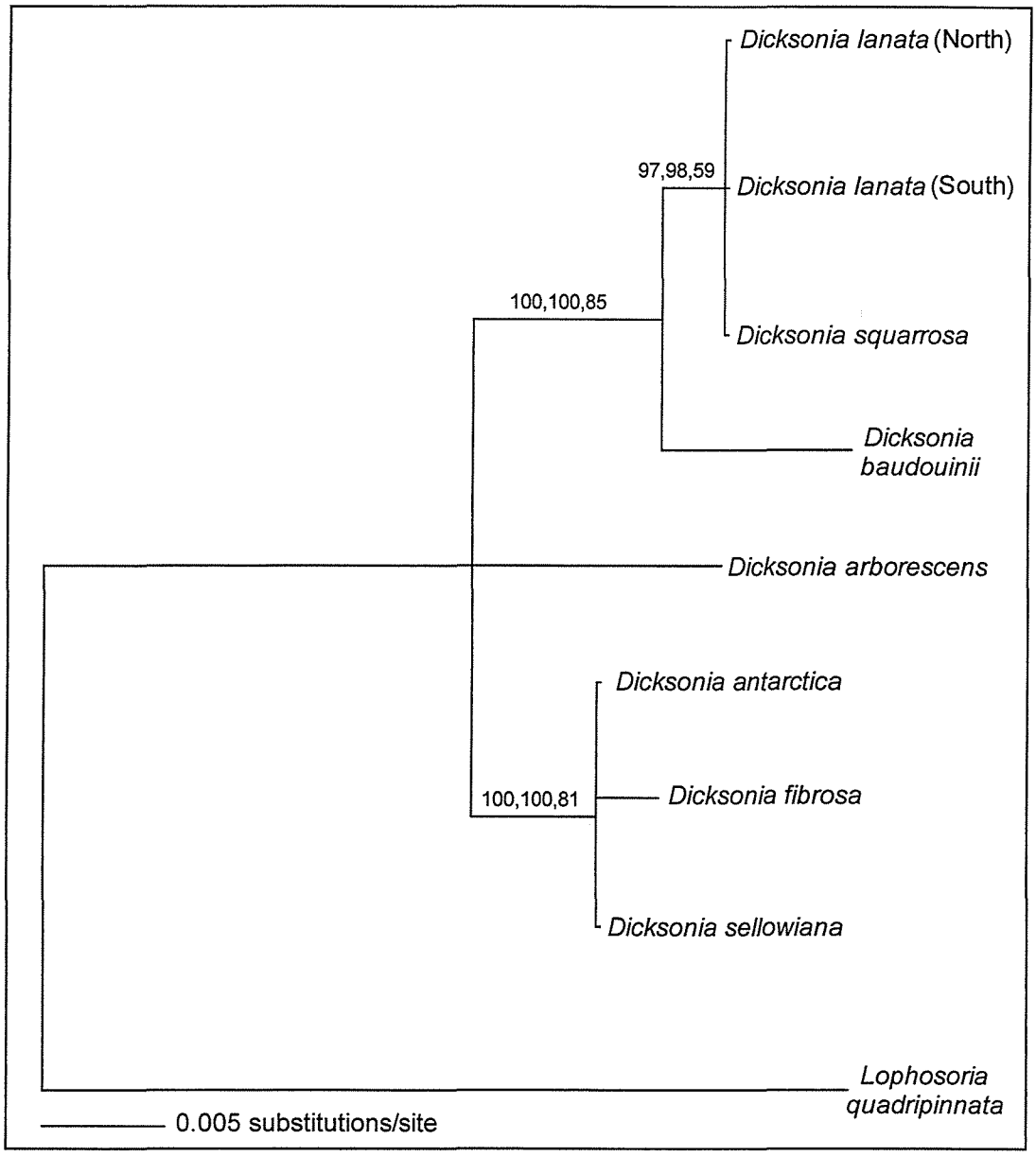

Figure 4.3 Quartet Puzzling Tree for *Dicksonia trnL-trnF* Sequences: The optimality criterion used is Maximum Likelihood with the GTR model. The three numbers on the splits indicate: 1) Puzzle values from the ML tree, 2) Puzzle values from an MP tree and 3) Bootstrap values from an NJ tree. For equal values only a single number is shown. Support values for clustering of the three groups is as follows: *D. antarctica* group with *D. arborescens*:: 31,98,61; *D. antarctica* group with *D. lanata* group: 36,<5,9; and *D. arborescens* with *D. lanata* group: 33,<5,20.

In all trees *Dicksonia arborescens* formed the basal branch of the *Dicksonia* group. One of the *Dicksonia antarctica* sequences, from Wolf *et al.* 1994, appeared to be quite different from the other two *D. antarctica* sequences, clustering with the

reticulate spore group, possibly due to sequencing errors. The branch leading to *D. baudouinii*, *D. lanata* and *D. squarrosa* was relatively long.

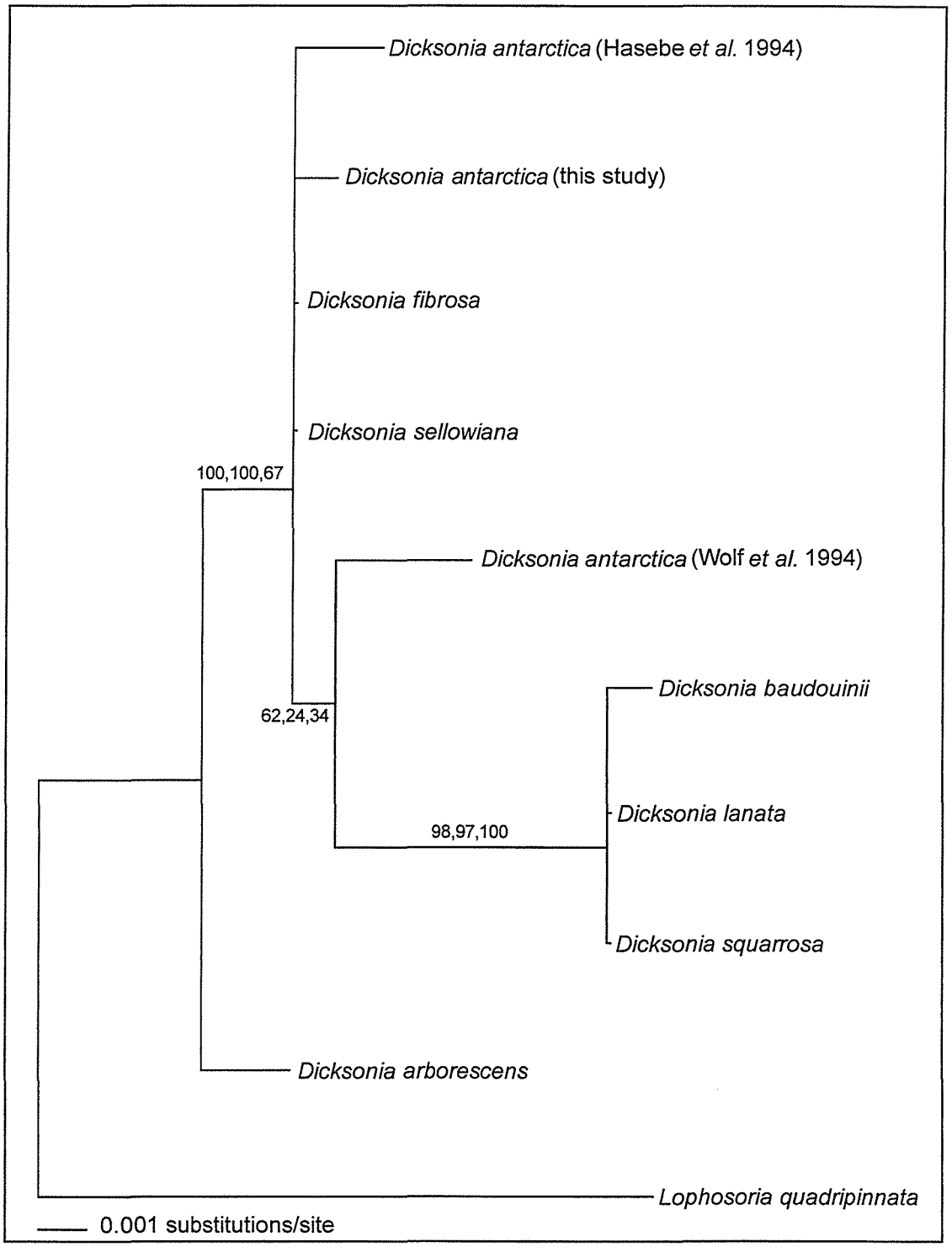

Figure 4.4 Quartet Puzzling Tree for *Dicksonia rbcL* Sequences: The optimality criterion used is Maximum Likelihood with the GTR model. The three numbers on the splits indicate: 1) Puzzle values from the ML tree, 2) Puzzle values from an MP tree and 3) Bootstrap values from an NJ tree. For equal values only a single number is shown.

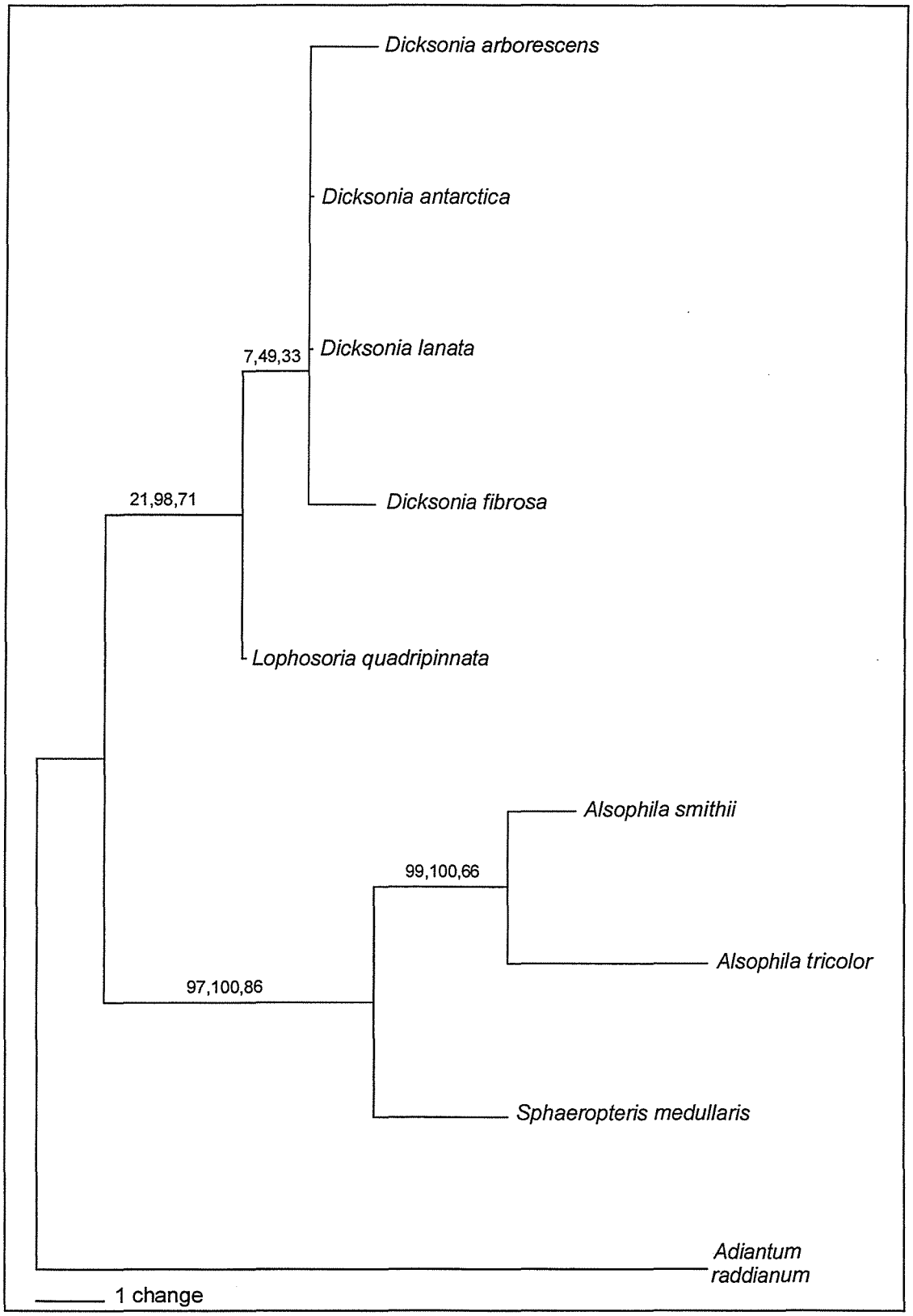

Figure 4.5 Quartet Puzzling Tree for *18S* Sequences: The optimality criterion used is Maximum Parsimony with the GTR+I+G model. The three numbers on the splits indicate: 1) Puzzle values from an ML tree, 2) Puzzle values from the MP tree and 3) Bootstrap values from an NJ tree. For equal values only a single number is shown.

18S

Trees were reconstructed for the *18S* data using the GTR+I+G model under ML Quartet Puzzling, and Neighbour Joining, from 1671 unambiguous sites. A Maximum Parsimony QP tree was also built for comparison.

Figure 4.5 shows the MP tree. The relationships among the *Dicksonia* species are unresolved due to lack of signal. However, there is sufficient signal to distinguish all four ingroup genera included in this analysis: *Alsophila, Dicksonia, Lophosoria* and *Sphaeropteris*.

4.1.8 *Dicksonia lanata* AFLP

AFLP was performed on the five Northern and eight Southern populations, as well as three *D. squarrosa* outgroup populations. Initial AFLP gels were produced using duplicate extractions from all samples. Several test gels showed the method to be robust, with duplicates giving identical patterns. Only a single extraction from each sample was used in the final gels, with one duplicate to continue to check for robustness. This allowed two sets of the same samples to run on a gel for different lengths of time (e.g. 2 hours and 3 hours). Long runs gave good separation of bands at the top of the gel, while shorter runs were useful for visualising bands in the lower portion of the gel.

Ultimately, four successful gels were produced, using the primer combinations Eco-ATA/Mse-CAG, Eco-ATA/Mse-CTG, Eco-AAT/Mse-CAG and Eco-AGC/Mse-CTG. The Eco-AAT/Mse-CAG gel produced the most informative loci, at 96, while the Eco-AGC/Mse-CTG gel produced the fewest (20), and had smears in all the *Dicksonia squarrosa* samples, indicating some degradation of the DNA. The Eco-ATA/Mse-CTG gel produced 57 loci, and the Eco-ATA/Mse-CAG gel 56, resulting in a total of 229 loci from all four gels.

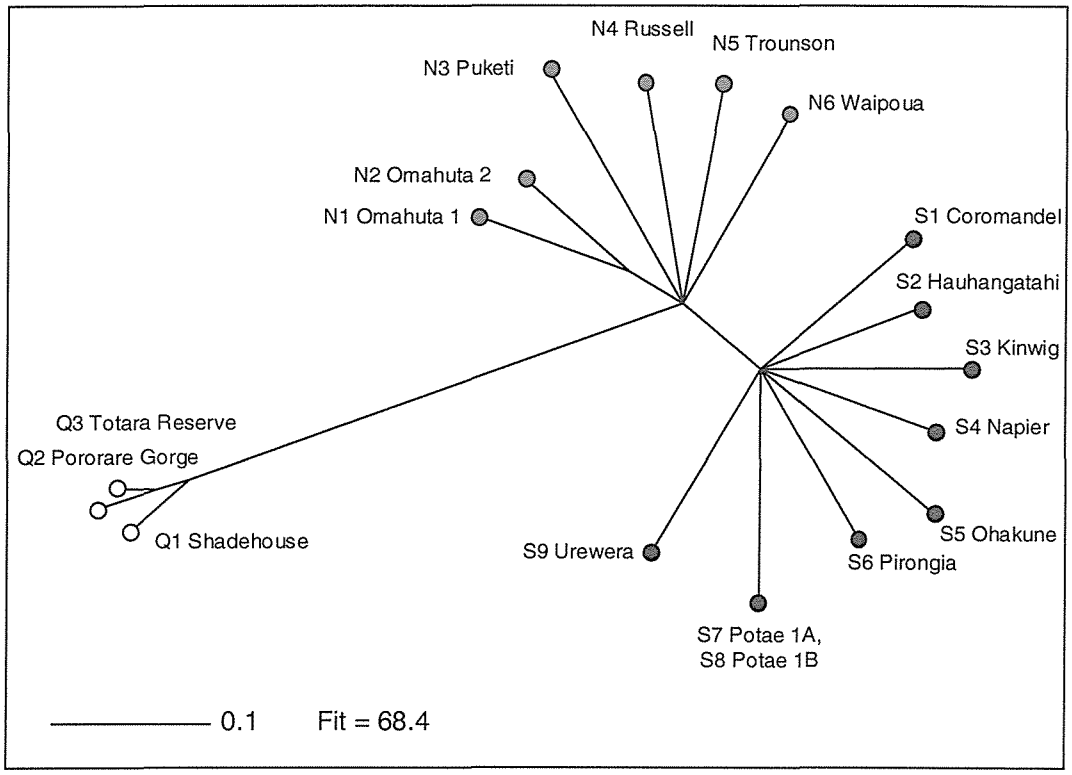

Figure 4.6 AFLP Splits Graph: results from split decomposition on AFLP data from *Dicksonia lanata* populations. Three *Dicksonia squarrosa* populations were included as outgroups. *Dicksonia lanata* Northern form = red, *Dicksonia lanata* Southern form = blue, *Dicksonia squarrosa* = yellow. Least squares edge fitting is on.

Binary coded data from the AFLP gels was analysed in both PAUP* and SplitsTree. In PAUP* trees were built using Neighbour Joining (NJ), and bootstrapping with Maximum Parsimony (MP) and Mean Character Distance as the optimality criteria. These graphs may be found in Appendix 4.3. The NJ tree showed long edges to individual populations with relatively short edges creating population groupings. The split between the two forms was evident, but not as long as the edges of individual populations. Bootstrapping with MP showed approximately 80% support for the North-South split. No internal relationships within these groups were well supported. The Distance bootstrap consensus tree was similar, with higher support for the North-South split (95%), and support for a few internal relationships.

The splits graph shown in Figure 4.6 was produced from all 229 characters. Edge lengths were optimised using a least squares criterion. The only internal relationship appeared to be from two individuals of the Omahuta population. As with the NJ tree from PAUP* the edge separating the two forms was short compared to the external edges.

4.2 *Dicksonia*

Splits graphs of all three markers showed the sequence data to be tree-like and suitable for phylogeny reconstruction. In evaluating nested models of sequence evolution, *rbcL* was best estimated by either the Kimura 2-parameter (1980) or GTR model. This contrasted with results from Chapter 3, in which *rbcL* evolution was best estimated by more complex models, for more deeply diverged taxa. With only *Dicksonia* and *Lophosoria* included, evolution of the data could be described by simpler models. This is presumably due to the extent of complexity of the underlying substitution process becoming more obvious when more highly diverged sequences are compared. For the *18S* marker, complex models incorporating an estimate of invariable sites and a gamma distribution of variable sites were found to better fit the data. This is expected, as *18S* is known to contain highly conserved as well as variable regions (e.g. Soltis *et al.* 1997). For the *trnL-trnF* spacer, the HKY, Fel84 and GTR models were found to be most appropriate. This is also as expected, as this is a non-coding sequence in which all bases should have equal substitution properties due to lack of functional constraint.

4.2.1 Three lineages

Results from the two chloroplast markers used in this study suggest three major groups within the genus *Dicksonia* (see Figures 4.1, 4.2, 4.3, 4.4). These three groups correspond to three spore types that have been observed (Large & Braggins 1991, Q. B. Cronk pers. comm.) as mentioned in the introduction. The first of these groups, the granulate or 'smooth', spore group is represented in this study by *D. antarctica*, *D. fibrosa* and *D. sellowiana*. The tuberculate, or 'knobbled', spore group is represented by *D. baudouinii*, *D. lanata*, and *D. squarrosa*. The final group, the 'intermediate' spore group is represented by a single species worldwide, *D. arborescens*.

Results from the *trnL-trnF* spacer under Quartet Puzzling with the GTR model suggest that the three spore types form a trichotomy (Figure 4.3), while QP with Maximum Parsimony, and Neighbour Joining suggest that *D. arborescens* is more closely related to the smooth spore group. Also, the *rbcL* data indicates that *D.*

arborescens may be ancestral (Figure 4.4). However, these conflicting conclusions are based on low numbers of changes, particularly in *rbcL* and are possibly not reliable. In the *trnL-trnF* splitsgraph (Figure 4.1) the branch lengths of the three groups are approximately equal, suggesting the three groups have probably diverged at approximately the same time.

Although the genus *Dicksonia* is known to be ancient (at least 140 mya, e.g. Van Konjinenberg-Van Cittert 1989), the relative branch lengths of the trees presented here suggest that the species observed within the genus cannot be this old. *D. arborescens* of St. Helena Island can be assumed to have a minimum age of 9 million years, as the island was only formed around this time. Furthermore, fossils of *D. arborescens* have been confirmed from 9 million year old deposits (Q. B. Cronk pers. comm.). As the three observed lineages appear to have diverged at approximately the same time, then they are probably at least 9 million years old. The earliest *Dicksonia* fossils are from the Jurassic (213 - 144 mya) and these appear to be similar to the granulate spore type observed in modern species such as *D. fibrosa* and *D. antarctica*. These spores are harder to identify, as they are less distinctive than the tuberculate type and similar to spores from some of the other Dicksoniaceae genera. Tuberculate spores are much easier to identify as being from *Dicksonia*, due to their distinctive architecture. These are not observed in the fossil record until the Tertiary (65 - 2 mya). Fossil *Dicksonia dissecta* from Australia is of this spore type, and has been found in early Oligocene (38 - 24.6 mya) strata. It has been suggested (Jordan *et al.* 1996) that the divergence between these two spore architectures occurred late in the Paleocene (65 - 54.9 mya). From the available fossil evidence the divergence between the three modern spore types has probably occurred in the range of 65 - 9 million years ago. Low variability in the DNA sequence argues towards the recent end of this range.

4.2.2 Recent Speciation

The low level of sequence variation over all three markers used in this study suggests that the species within each of these three lineages are more recent. In the *trnL-trnF* spacer the variability between the three sequenced members of the granulate spore group, *D. anarctica*, *D. fibrosa* and *D. sellowiana*, is only a single base change (in *D.*

fibrosa). In the *rbcL* data (excluding *D. antarctica* from Wolf *et al.* 1994) only 2 bp changes are observed. This suggests speciation may have occurred within this group as recently as the Quartenary (2 - 0 mya). Within the reticulate group, *D. lanata* and *D. squarrosa* are very close, separated by a single indel, again arguing for recent speciation. *Dicksonia baudouinii* is more diverged from these two species, and appears to have split from them somewhat earlier. Many more species are observed within this group compared with the smooth spore group. Unfortunately, no other material was available from this spore group (e.g. from Malesia or Juan Fernandez), so it is under-represented here. *Dicksonia arborescens* is the sole representative of the intermediate spore group, and is quite geographically distinct from the other two spore groups. How it arrived on St. Helena island remains a mystery. Based on the *trnL-trnF* data it may be more closely related to the granulate spore group than the tuberculate group.

The three lineages may have been geographically isolated at some point and dispersed across the Southern Hemisphere resulting in new species. Particularly in the granulate spore group, which are found in Australia, New Zealand and South America, long-distance dispersal has probably played a major role in their spread and subsequent speciation.

4.2.3 *Lophosoria*

Data from the *trnL-trnF* and *18S* markers consistently show *Lophosoria* to be closely related to *Dicksonia*, supporting previous *rbcL* data. This would place it firmly in the Dicksoniaceae (if the family is retained). However, *Lophosoria* produces a very short branch from the *Dicksonia* lineage in the *rbcL* data (which violates a molecular clock, see 3.1.4). Similarly in a combined *Dicksonia-Cyathea trnL-trnF* splits graph *Lophosoria* also shows a relatively short branch (data not shown). There is insufficient variability to draw any conclusions from *18S*, but the chloroplast markers suggest that *Lophosoria* does not conform to a molecular clock model. This may be explained by a hybrid origin for *Lophosoria*, or possibly the chloroplast is evolving much slower than in the other tree fern taxa.

4.3 *Dicksonia lanata*

Dicksonia lanata is an interesting case within *Dicksonia* and the tree fern clade as a whole, due to the presence of two distinct growth forms within the species complex. Due to the length of the life-cycle of these plants a morphological study into whether these plants hold their form when transplanted would be impractical (i.e. whether or not this is in response to environmental conditions). However, anecdotal evidence suggests that they do retain their form when transplanted (plants have been grown in cultivation in Auckland for seven years, S. Van der Mast pers. comm.). Morphologically then, there is a clear difference between the two forms. One, the Northern form, has an erect trunk to 2 m, while the other, the Southern, possesses a creeping rhizome. In an attempt to assay genetic difference between these two forms Amplified Fragment Length Polymorphism (AFLP) was used. This technique is described in detail in section 2.4.

Once initial problems in generating the AFLP gels were overcome the first gel to be scored was produced. On casual inspection the bands on the gel between the Northern and Southern forms appeared to be essentially random. No clear banding patterns splitting the two groups were evidenced, although the outgroup species used, *D. squarrosa* was obviously separate from the *D. lanata* samples. Eventually four gels were generated using different primer combinations and scored, resulting in data from 229 loci. When coded into a nexus file for analysis by SplitsTree and PAUP* it became apparent that there is considerable evidence for genetic differentiation between the Northern and Southern forms. In contrast, little internal branching structure within either the Northern or Southern groups was evident under split decomposition and in bootstrapped bifurcating trees reconstructed in PAUP*.

When the data was bootstrapped in PAUP* using both Maximum Parsimony and Distance-based methods the only signals in the data that were well supported were: (1) the split between *D. lanata* and *D. squarrosa* (consistently at 100%), (2) two duplicate extractions of the same plant from the Ruahine ranges (again at 100%), and (3) the split between the Northern and Southern forms (with approximately 80% bootstrap support).

4.3.1 Two Species?

The appearance of the split between the Northern and Southern forms is suggestive of some genetic isolation between them. However, the seemingly random pattern of bands observed suggests that genetic isolation may not be complete. This contrasts with a similar experiment by Perrie *et al.* (2000) in *Polystichum richardii* (W. J. Hooker) J. Smith, where a morphological variation within the species complex was observed. In the case of *P. richardii*, when AFLP experiments were performed banding patterns separating the two observed morphological types were readily apparent.

Whether these two forms represent a geographic cline, with the split an artefact of insufficient sampling, or whether the beginnings of speciation are being observed remains uncertain. The fact that the two forms are spatially separated with no overlap between them, and a degree of genetic isolation between them may argue towards speciation. However, the split between the two forms is very short when compared with the external edges, and especially short when compared with the split between *D. lanata* and *D. squarrosa* (its closest New Zealand relative). This suggests that these two forms are only in the beginnings of speciation. Therefore, recognition at sub-specific level may be appropriate.

4.3.2 Origins of the Two Growth Forms

Two hypotheses could explain the origins of the two forms of *D. lanata*. The tectonic hypothesis attempts to account for the current distribution of plants within New Zealand (McGlone 1985) by suggesting that the land form of New Zealand has undergone considerable change in the last 25 million years. In the Pliocene (5 million years ago), Northland became an archipelago with a narrow strait through the Auckland area. Populations of *D. lanata* may have become separated during this time, leading to the current variations in morphology. In this case the hypothesis would seem to be discounted by the similarity between *D. squarrosa* and *D. lanata* observed from DNA sequencing work. This suggests these two species may be as recently derived as the last 2 million years. The timeframe of the tectonic hypothesis is the last

5 million years, which is clearly far too long if the species are only very recently derived.

The glacial refugia hypothesis predicts that plants retreated to refugia during the peak cold of the Last Glaciation (McGlone 1985). They spread out from these refugia when conditions became more suitable, about 10,000 - 14,000 years ago. These refugia were scattered along the coastal ranges of the South Island and north of latitude 38 - 39 °S. It is possible that the two sub-types of *D. lanata* were confined to separate refugia during this period. This hypothesis is generally poorly supported by correlations of plant distributions with ice-free areas (Pole 1994) but seems more likely to explain the two forms of *D. lanata* due to their seemingly recent origins. The two forms may be due to separation during the last ice age, or perhaps two or three ice ages ago, probably not more than 30,000 years ago.

The two observed forms may represent climatic adaptations post-separation. The Northern form, which is found only within kauri forests, is much less susceptible to wind and cold due to the protective surrounding forest. As such, it is able to grow taller while sheltered by the forest. The Southern form is found in generally higher altitude sub-alpine terrain with little forest cover. This form may have reduced or lost its trunk in response to this environment. Being much closer to the ground may provide protection from wind and frost.

CYATHEACEAE

5.1 Results

Phylogenetic analysis of the New Zealand members of the Cyatheaceae from DNA sequence data is reported in this chapter.

5.1.1 DNA Extractions

Extractions of Cyatheaceae samples were in general more successful than those from the genus *Dicksonia*, as the frond tissue is much softer. All samples were extracted from fresh tissue (rather than dried) which also improved extraction yield. However, some Cyatheaceae samples (e.g *Sphaeropteris medullaris*, *S. tomentosissima, Cyathea delgadii*) appeared to contain large amounts of polysaccharide which was present at the bottom of the tube after the isopropanol step. This interfered with attempts to isolate the DNA. In these cases the sample was spun down gently and the solution was poured off, leaving a pellet of DNA and polysaccharide. This pellet was then redissolved in CTAB solution by gentle heating, and then chloroform was added and the extraction procedure repeated. The resulting DNA appeared white and was free of tissue fragments.

5.1.2 PCR and Sequencing

rps4 & trnL intron

The chlorplast *rps4* gene and *trnL* intron have been successfully used as phylogenetic markers in several studies (original references in Nadot *et al.* 1994, Taberlet *et al.* 1991). PCR of these markers was not attempted with any of the Cyatheaceae samples, as earlier attempts to amplify the region in *Dicksonia* were unsuccessful.

trnL-trnF spacer

The *trnL-trnF* spacer is a non-coding region located between two chloroplast tRNA genes which has also been successfully used as a phylogenetic marker. After trials on members of the genus *Dicksonia* this marker was also used on Cyatheaceae. The region amplified successfully with all species from of this group. PCR was carried out on eleven species; the six native *Alsophila* species, four *Sphaeropteris* species and a single *Cyathea.* Bands on a 1% agarose gel between the *Alsophila* species and some *Sphaeropteris* and *Cyathea* species could be distinguished by size with a c. 50 bp difference in size. The PCR products appeared to be approximately 400 bp long in *Alsophila* and *S. medullaris*, and 350 bp in *Sphaeropteris excelsa*, *S. robusta*, *S. tomentosissima* and *C. delgadii.*

Sequencing was performed on all eleven species using the two primers TabE and TabF, to obtain the entire c. 400 bp sequence. The sequences from some of these species were ambiguous in some regions due to the presence of homopolymers within the sequences. The polymerase had difficulty sequencing through a string of c. 10 C's followed by a string of c. 10 A's in *Alsophila cunninghamii* and *A. kermadecensis*. Similarly in *A. milnei* and *A. tricolor* a long repeat of 10+ T's also disrupted sequencing. The sequences become very noisy at the 3' end of these repeats in the both the forward and reverse directions, resulting in readable sequence in only one direction on either side of the repeats (missing approximately 15 - 30 bp at the ends). Although the single direction sequences were clearly readable, it would be preferable to have full sequence in both directions. The exact length of the repeats is uncertain due to this noise. Attempts to improve sequencing by using the dGTP kit, and by using the BigDye terminator with increased time for denaturing and annealing were unsuccessful.

rbcL

Four species were sequenced for *rbcL*, *A. smithii*, *A. tricolor*, *S. excelsa* and *S. medullaris*. PCR products were approximately 1.3 kb in length. Preliminary sequencing was performed using the aF and cR primers. This was sufficient to provide a single strand read for most of the c. 1300 bp sequence. Three additional

primers were used to obtain a full sequence for both the forward and reverse strands (422F, 961F and 579R: see Appendix 2.3 for primer sequences). The complete open reading frame was 1320 bp in length. Species of *Sphaeropteris* were sequenced using these five primers. However, one of the primers (developed for use with *Polystichum*) failed to produce readable sequence for *Alsophila* and *Dicksonia*. One base pair at the start of the sequence differed from that of the primer sequence in these two genera. A new primer that exactly matched the *Alsophila* and *Dicksonia* sequences (dic579R) was developed and used successfully.

ITS

The nuclear *ITS* region consists of two short spacers (*ITS-1* and *ITS-2*) either side of the *5.8S* rRNA gene, and is present in multiple copies within the genome. Initial trials in *Dicksonia* with *ITS* were unsuccessful, repeatedly producing multiple bands, consequently further PCR of this marker was abandoned.

18S

The *18S* gene is also found in the nuclear genome in multiple copies together with the *ITS* region. The primers 18sF and 18sR were used to amplify the *18S* region in *Alsophila smithii*, *A. tricolor* and *Sphaeropteris medullaris*, a result not surprising given the highly conserved nature of the gene. PCR products on agarose were approximately 1.8 kb in length. These three species were sequenced using four primers, 18sF, 18sF2, 18sR and 18sR2m, giving overlapping sequence from both strands only for the middle c. 600 bp. Sequence determination either side of this was made only for one strand. However, this sequence showed little or no variation. Due to the low variability observed it was not considered worthwhile to continue sequencing the second strand either side of the middle 600 bp region.

5.1.3 DNA Sequence Alignments

Positional homology among DNA sequences was inferred using the progressive multiple sequence alignment procedure implemented in Clustal X (1.8). These alignments can be found in Appendix 5.1.

trnL-trnF

The complete alignment of the *trnL-trnF* spacer region for the Cyatheaceae samples may be found in the appendix. This alignment was 443 bp long. The longest individual sequence was *A. tricolor*, at 407 bp. The shortest sequence was from *A. kermadecensis* (sequence was missing at the 5' and 3' ends). The shortest complete sequence was from *S. excelsa*, at 345 bp. Several single base pair changes and indels were present between these species. In addition, all of the sequences contained three mononucleotide repeats, A_n and C_n near the beginning and T_n near the middle. These were variable in length, being longer in the *Alsophila* species. The *Cyathea delgadii* sequence was identical to that of *S. excelsa*, and it was assumed that this sample had been mislabelled. *Sphaeropteris tomentosissima* was also almost identical to *S. excelsa* and *S. robusta.*

Due to frequent gaps and a high level of variability, sequences from *Lophosoria quadripinnata* and *Dicksonia* sp. could not be reliably aligned with the Cyatheaceae sequences.

rbcL

The *Alsophila* and *Sphaeropteris rbcL* sequences were part of an alignment that included all available sequences for the seven families of the 'tree fern clade', with *Azolla caroliniana* included as an outgroup. Discussion of this alignment may be found in the Section 3.1.

18S

The three Cyatheaceae sequences obtained for the *18S* marker were aligned with four *Dicksonia* species, *Lophosoria*, and a sequence from *Adiantum raddianum* as an outgroup. The sequence variability between all of these species was very low. There were 2 bp differing between the two *Alsophila* sequences, and only 6 bp variable between *Alsophila* and *Sphaeropteris*. Phylogenetic analysis of the *18S* marker is included under Chapter 4.

Marker + Taxa	No. Seq.	Length Range	Aligned Length	Single bp Changes	Indels	Percent Variability	Base Composition A	C	G	T	% A-T
***trnL-F* (chloroplast)**											
Alsophila	5	331 - 407	443	10	4	3.16%	0.267	0.283	0.133	0.317	58.33
Sphaeropteris	4	345 - 406	443	8	6	3.16%	0.100	0.400	0.000	0.500	60.00
Cyatheaceae[1]	9	331 - 407	443	25	10	7.90%	0.177	0.268	0.201	0.354	53.11
***rbcL* (chloroplast)**											
Alsophila	2	1320	1334	13	0	0.97%	0.231	0.231	0.192	0.346	57.69
Sphaeropteris	5	1265 - 1320	1334	20	0	1.50%	0.160	0.370	0.130	0.340	50.00
Cyatheaceae[1]	7	1265 - 1320	1334	45	0	3.37%	0.216	0.283	0.197	0.305	52.06
Cyatheaceae+*Hymenophyllopsis*	9	1265 - 1321	1334	67	0	5.02%	0.234	0.302	0.169	0.295	52.90
***18S* (nuclear)**											
Alsophila	2	1704 - 1742	1811	4	0	0.22%	0.250	0.250	0.125	0.375	62.5
Cyatheaceae[1]	3	1691 - 1742	1811	6	0	0.33%	0.250	0.292	0.125	0.333	58.30
Dicksonia+Cyatheaceae	7	1691 - 1742	1811	12	0	0.66%	0.224	0.367	0.102	0.306	53.06

Table 5.1 Summary of DNA Sequence Marker Data for Cyatheaceae: No. Seq. = number of sequences in specified group; Length Range = range of sequence lengths within specified group; Aligned Length = aligned sequence length including gaps; Single bp changes = number of sites with single nucleotide substitutions within specified group; Indels = number of insertions or deletions present within specified group; Percent Variability = estimate of sequence variability within specified group, calculated as (Single bp changes + Indels) / Aligned length. Cyatheaceae[1] = *Alsophila*, *Sphaeropteris*. For a full list of species sequenced for each marker refer to alignments in Appendix 3.1 and 5.1.

5.1.4 Usefulness Of Sequence Data For Tree Building

Split decomposition was used to investigate the usefulness of the gathered sequence data for inferring evolutionary relationships within the Cyatheaceae. For the *trnL-trnF* spacer region, exclusion of missing and ambiguous characters left 243 for analysis. The resulting splits graph showed some reticulation due to the presence of *A. smithii* but was otherwise tree-like (Figure 5.1).

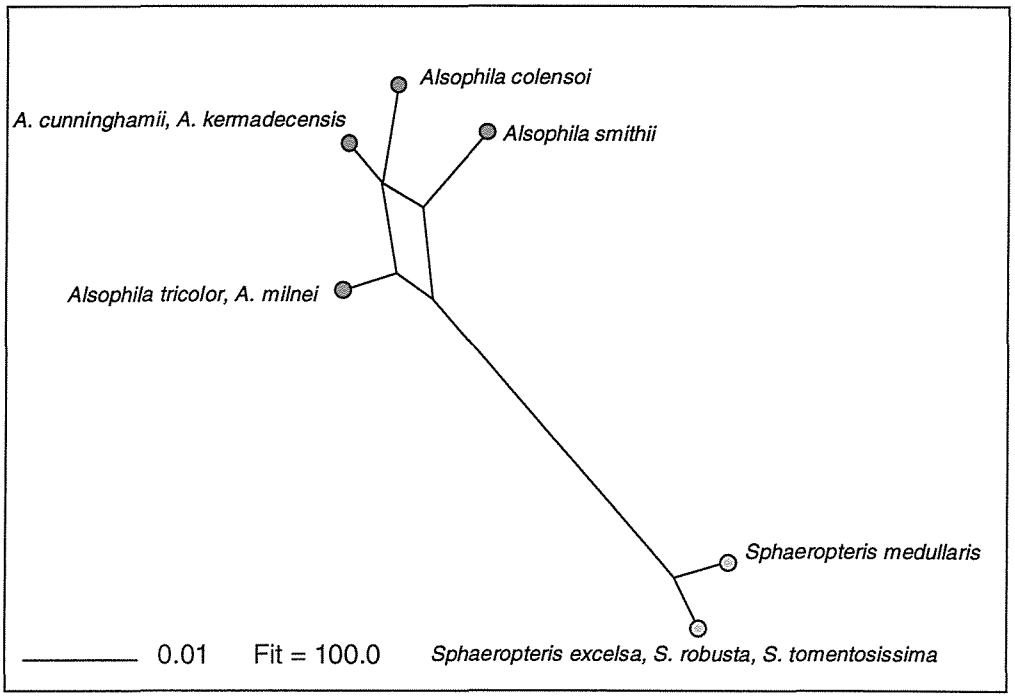

Figure 5.1 Cyatheaceae *trnL-trnF* Splits Graph: New Zealand *Alsophila* species, *Sphaeropteris* from NZ, Australia, New Guinea.

As before, the *rbcL* data had already been shown to be tree-like (Figure 3.1) and suitable for tree building.

5.1.5 Choosing A Substitution Model For Tree Building

Nested models of sequence evolution were again evaluated for their fit to the data for use in tree building. Tests of fit were made on a Neighbour Joining tree assuming "p" (Hamming) distances. Results of this analysis are shown in Table 5.2. Empirical base compositions were assumed when estimating other parameters.

For the *trnL-trnF* spacer region the HKY, Fel84 and GTR models were found to be most appropriate for the data. The HKY+I+G, Fel84+I+G and GTR+I+G models best approximated the *rbcL* data.

Cyatheaceae *trnL-F*									
	HKY	**ΔW**	**χ2**	**Fel84**	**ΔW**	**χ2**	**GTR**	**ΔW**	**χ2**
+G+I	451.000			450.994			445.794		
+I	451.055	0.108	3.841	450.994	0.000	3.841	445.803	0.017	3.841
-	451.130	0.150	3.841	451.132	0.275	3.841	445.870	0.134	3.841
K2P	458.430	14.600	9.488	458.430	14.595	9.488	458.430	25.119	9.488
JC69	462.579	8.299	3.841	462.579	8.299	3.841	462.579	8.299	3.841
Cyatheaceae *rbcL*									
	HKY	**ΔW**	**χ2**	**Fel84**	**ΔW**	**χ2**	**GTR**	**ΔW**	**χ2**
+G+I	2081.682			2081.571			2079.438		
+I	2083.942	4.520	3.841	2083.826	4.510	3.841	2081.758	4.639	3.841
-	2084.261	0.637	3.841	2084.144	0.637	3.841	2082.079	0.642	3.841
K2P	2087.633	6.745	9.488	2087.633	6.978	9.488	2087.633	11.108	9.488
JC69	2101.740	28.213	3.841	2101.740	28.213	3.841	2101.740	28.213	3.841

Table 5.2 Likelihood Ratio Tests for the Cyatheaceae: Three series of models (HKY, Fel84, GTR) were evaluated for two different markers, *trnL-trnF* and *rbcL*. ΔW values were compared with χ2 values to test for significance. For the *trnL-trnF* marker the HKY, Fel84 and GTR models gave a best fit to the data. For *rbcL* the HKY+I+G, Fel84+I+G and GTR+I+G models were best.

Cyathea *rbcL*		**Tree Score**			
Tree	**Root**	**No Clock**	**Clock**	**ΔW**	**χ2**
QP-ML	*Hymenophyllopsis*	2251.679	2259.985	16.612	14.067
QP-ML	*Alsophila*	2251.679	2260.518	17.676	14.067
QP-ML	*Sphaeropteris*	2251.679	2260.518	17.676	14.067
NJ	*Hymenophyllopsis*	2251.679	2259.985	16.612	14.067
NJ	*Alsophila*	2251.679	2260.518	17.676	14.067
NJ	*Sphaeropteris*	2251.679	2260.518	17.676	14.067
Cyathea *trnL-trnF*		**Tree Score**			
Tree	**Root**	**No Clock**	**Clock**	**ΔW**	**χ2**
QP-ML	*Sphaeropteris*	445.846	447.362	3.032	16.919
NJ	*Sphaeropteris*	445.846	447.362	3.032	16.919

Table 5.3 Molecular Clock Tests for the Cyatheaceae: Two trees were evaluated for their fit to a molecular clock, a Quartet Puzzling Maximum Likelihood (QP-ML) tree and a Neighbour Joining tree (NJ), for two different markers, *trnL-trnF* and *rbcL*. ΔW values were calculated from comparison of tree scores with or without a molecular clock enforced, and compared with χ2 values to test for significance. The *rbcL* tree was rooted in several different positions. Both markers passed the test, indicating that the sequences are evolving in a clock-like fashion.

Molecular clock tests were performed on the *rbcL* and *trnL-trnF* data for the Cyatheaceae (Table 5.3). These were made by comparing the scores of the best trees constrained and unconstrained for rate variation along branches. Both markers passed indicating the sequences are evolving in a clock-like manner.

5.1.6 Evolutionary Trees

Evolutionary trees were reconstructed using PAUP* 4.0b5. For the *trnL-trnF* data phylogenies were reconstructed using Quartet Puzzling (QP) and Neighbour Joining (NJ) under the GTR model with the parameter settings estimated in 5.1.5. In addition, a Maximum Parsimony QP tree was reconstructed for comparison. Due to the lack of a suitable outgroup, the trees were rooted at the midpoint. The GTR QP tree is shown below with support for edges from each tree indicated.

This tree showed *A. milnei* and *A. tricolor* as a basal branch of the *Alsophila* lineage, with *A. smithii* as the next branch, followed by *A. colensoi*. *Alsophila cunninghamii* and *A. kermadecensis* were terminal branches of this lineage. In the *Sphaeropteris* lineage *S. medullaris* was basal, with an internal group of *S. excelsa*, *S. robusta* and *S. tomentosissima*.

For the *rbcL* data, trees were reconstructed in a similar manner, but with the GTR+I+G model used for Quartet Puzzling and Neighbour Joining. Parameter settings estimated in 5.1.5 were utilised. A Maximum Parsimony QP tree was again included for comparison.

The resulting tree was identical in topology for these taxa to the *rbcL* tree shown in Figure 3.2. *Hymenophyllopsis* appears slightly more closely related to *Sphaeropteris* than *Alsophila*.

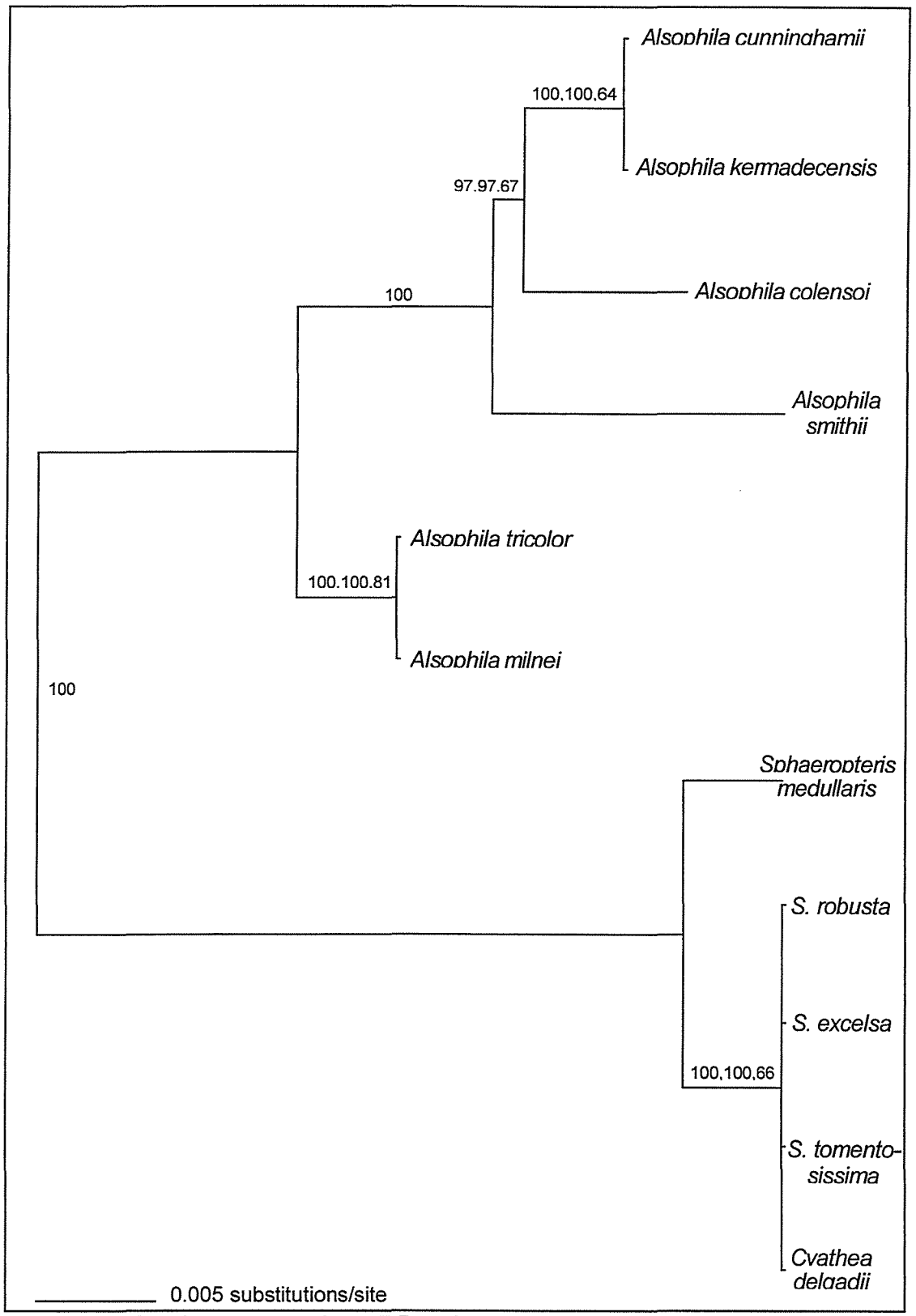

Figure 5.2 Cyatheaceae *trnL-trnF* Puzzle Tree: Built using Quartet Puzzling with Maximum Likelihood under a GTR model. Support values on splits indicate 1) ML puzzle values, 2) MP puzzle values, 3) NJ bootstrap values. A single number indicates all three are equal. Note: *Cyathea delgadii* proved to be a mislabelled *Sphaeropteris* (most likely *S. excelsa*).

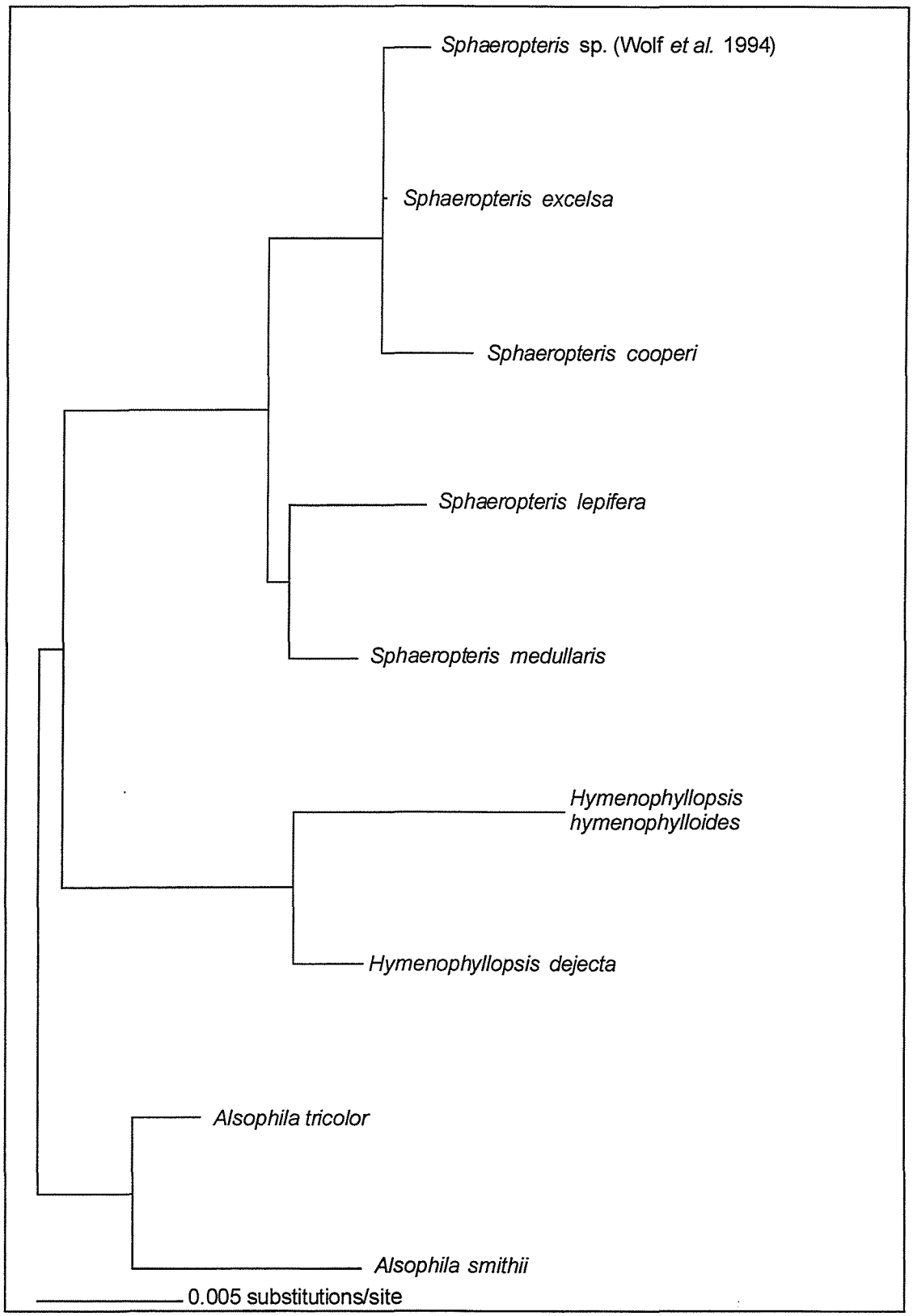

Figure 5.3 Cyatheaceae *rbcL* Puzzle Tree: Built using Quartet Puzzling with Maximum Likelihood under a GTR+I+G model. Support values on splits indicate 1) ML puzzle values, 2) MP puzzle values, 3) NJ bootstrap values. A single number indicates all three are equal.

5.2 Discussion

Results from likelihood ratio tests on the *trnL-trnF* and *rbcL* data identified similar substitution models for *Dicksonia* and the Cyatheaceae. With the *trnL-trnF* spacer a GTR model was assumed for both the Cyatheaceae and *Dicksonia*. Base compositions were unequal. However, positional heterogeneity was not evident - as might be expected given that the spacer region is non-coding.

In contrast, the *rbcL* gene evolution in the Cyatheaceae was best approximated with the GTR+I+G model, consistent with it being a coding sequence with sites able to vary to differing degrees. For analysis of *rbcL* data in *Dicksonia* a simpler GTR model was sufficient.

Molecular clock tests indicated that the sequence markers *rbcL* and *trnL-trnF* are evolving in a clock-like fashion in the Cyatheaceae. For *rbcL*, there appears to be rate variation among the different lineages (see Table 3.3), but within the two lineages examined here (*Dicksonia* + *Lophosoria*, Cyatheaceae + *Hymenophyllopsis*) sequence evolution appears clock-like.

5.2.1 New Zealand Cyatheaceae

Recent studies (e.g. Conant *et al.* 1996) based on chloroplast restriction site data have provided new insight into the subgroups within this family as discussed in the Introduction (1.3.1). However, the New Zealand Cyatheaceae are not well represented in such studies. As such, work in this thesis represents the first molecular study covering the New Zealand Cyatheaceae.

The New Zealand Cyatheaceae have been considered to all be part of a single genus *Cyathea* after Holttum and Edwards (1983). This thesis follows one of the most recent classification systems (that of Lellinger, 1987), which most closely approximates results from Conant *et al.* 1996, that indicate three lineages within *Cyathea*. Under Lellinger's scheme the New Zealand species examined in this thesis would fall into two genera, *Alsophila* and *Sphaeropteris*, as reported in section 1.3. Data from all

three molecular markers sequenced in this study support this classification, although the *18S* tree is based on only a few nucleotide substitutions. The *trnL-trnF* spacer and *rbcL* trees both strongly support the existence of two lineages in New Zealand. If the classification scheme of Lellinger (1987, as used in this thesis) is accepted, the New Zealand species fall into two genera. For most species the nomenclature would require a simple substitution of the generic name *Cyathea* for either *Alsophila* or *Sphaeropteris*, retaining the specific name. However, *Cyathea dealbata*, New Zealand's silver fern, becomes *Alsophila tricolor*, which is the first available name for this species in the genus *Alsophila* (see Table 5.2 and Brownsey 1985), the earlier specific epithet "dealbata" having already been used in *Alsophila* (*A. dealbata* Presl).

Name after Holttum (1983)	Name after Lellinger (1987)
Cyathea colensoi (J. D. Hooker) Domin	*Alsophila colensoi* J. D. Hooker
Cyathea cunninghamii J. D. Hooker	*Alsophila cunninghamii* (J. D. Hooker) R. Tryon
Cyathea dealbata (G. Forster) Swartz	*Alsophila tricolor* (Colenso) R. Tryon
Cyathea kermadecensis W. R. B. Oliver	*Alsophila kermadecensis* (W. R. B. Oliver) R. Tryon
Cyathea medullaris (G. Forster) Swartz	*Sphaeropteris medullaris* (G. Forster) Bernhardi
Cyathea milnei J. D. Hooker	*Alsophila milnei* (J. D. Hooker) R. Tryon
Cyathea smithii J. D. Hooker	*Alsophila smithii* (J. D. Hooker) R. Tryon

Table 5.4 Name Changes for NZ Cyatheaceae: Revision of the New Zealand Cyatheaceae into genera in the sense of Lellinger (1987) is supported by molecular studies in this thesis and by those of Conant *et al.* (1994, 1996).

Data from the *trnL-trnF* spacer tree for the New Zealand Cyatheaceae supports many of the observations about the relationships of these species based on morphological characters. There is a long edge between *Alsophila* and *Sphaeropteris* consistent with the presence of two genera. *Sphaeropteris excelsa* and *S. robusta* are almost identical to each other, and somewhat more distant to *S. medullaris*.

Within *Alsophila*, *A. kermadecensis* and *A. milnei* were confirmed as island variants of the mainland species *A. cunninghamii* and *A. tricolor* respectively. The sequences of these two being nearly identical to the mainland species. These data confirm the presence of 4 lineages within the New Zealand *Alsophila* species, corresponding to the four mainland species: *A. colensoi*, *A. cunninghamii*, *A. smithii* and *A. tricolor*. *Alsophila tricolor* appears to be the most basal lineage, then *A. smithii*, with the other two more recent. Additional evidence from *rbcL* would have been useful due to the

microsatellite problem of the *trnL-trnF* locus, however, this was beyond the scope of this study.

Morphological vs. Molecular data

To investigate agreement between the morphological and molecular data, morphological characters were mapped onto a phylogenetic tree obtained from the *trnL-trnF* data (Figure 5.3). The *trnL-trnF* tree selected was the Quartet Puzzling tree using Maximum Likelihood with the GTR model. *Sphaeropteris excelsa*, *S. robusta* and *S. tomentosissima* were excluded from the tree as there was insufficient data to draw any conclusions about *Sphaeropteris*.

Frond hair and scale characteristics mapped onto the tree (Figure 5.3, A) provide some insights into the reliability of the *trnL-trnF* tree. *Sphaeropteris medullaris* has distinctive spiny-margined scales which are characteristic of the genus. Among the *Alsophila* species there is some variation in hair and scale morphology that provides useful information. In *A. milnei* and *A. tricolor* the fronds are covered in dense curly hairs, and have scales lacking terminal spines found mostly towards the base of the frond. The other four species possess similar hair and scale characters, supporting their positions on the tree. *Alsophila smithii* possesses red or white stellate hairs, and has scales lacking spines. *Alsophila colensoi*, *A. cunninghamii* and *A. kermadecensis* all possess scales terminating in single or stellate spines. Of these three, only *A. kermadecenis* lacks stellate hairs. These characters appear to support the *trnL-trnF* tree.

When mapped onto the tree indusial characters (Figure 5.3, B) do not show any significant groupings (other than the mainland-island combinations of *A. cunninghamii* and *A. kermadecensis*, and *A. milnei* and *A. tricolor*). Perhaps the only available information from this is a trend towards loss of indusium in the *Alsophila* clade, with the complete loss of indusia in *A. colensoi* representing the most derived state. The basal species *A. milnei* and *A. tricolor* possess fuller indusia, with a reduction in *A. smithii*, *A. cunninghamii* and *A. kermadecensis*, to a complete loss in *A. colensoi*. The indusial characters do not strongly support the tree, but also do not conflict with it.

When spore characters are mapped onto the tree (Figure 5.3, C) the ridged architecture observed in *Alsophila colensoi*, *A. cunninghamii* and *A. kermadecensis* forms a monophyletic group. The smooth spore of *A. smithii* is unique among the species. The spinulate spore type occurs in *A. milnei*, *A. tricolor* and *Sphaeropteris medullaris*. Although *Alsophila* and *Sphaeropteris* spores are similar, they are not necessarily identical. These species do not form a monophyletic group, but the basal position of *A. milnei* and *A. tricolor* in the *Alsophila* lineage indicate that this spore type may be ancestral between the two genera, or the result of convergent evolution. The observed spore types appear to support the topology of the *trnL-trnF* tree.

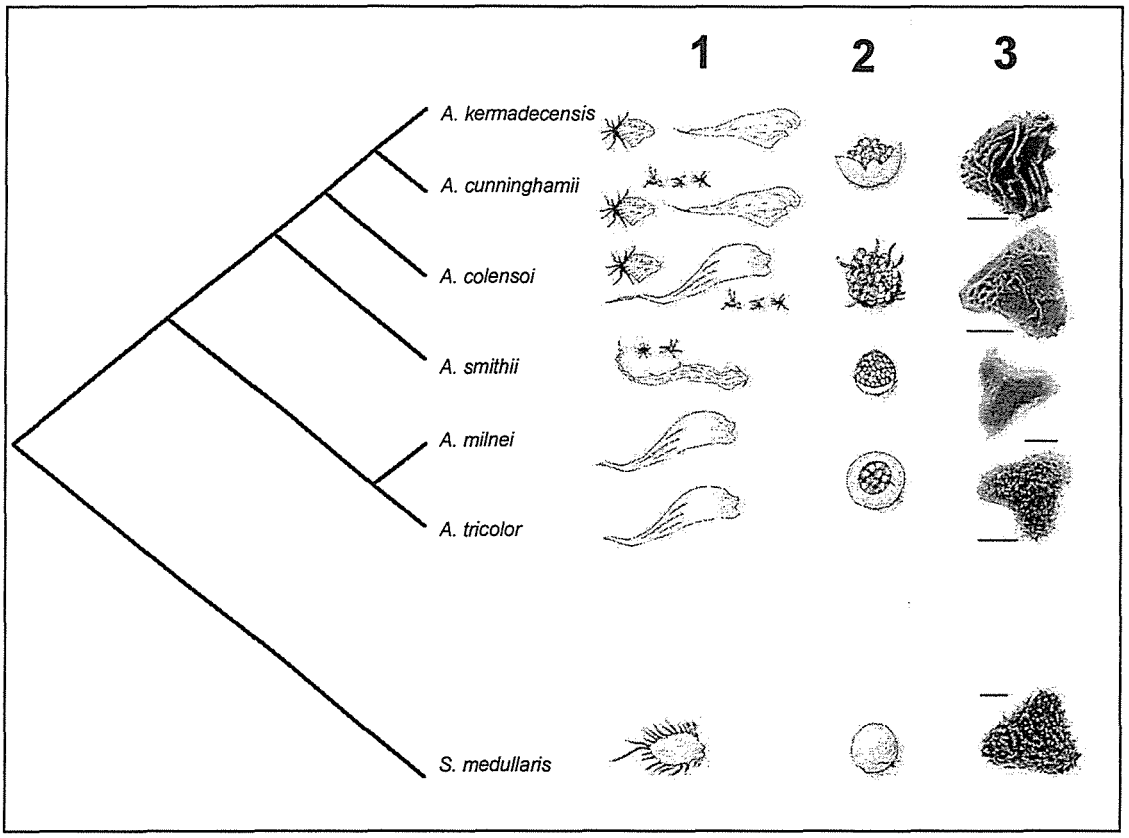

Figure 5.4 *trnL-trnF* Tree with Morphological Characters: *A.* = *Alsophila*, *S.* = *Sphaeropteris*. 1) Hair and scale morphology; 2) Indusium shape; 3) Spore morphology, scale bars in spore pictures are 15 µm. Frond hair, scales and indusium pictures taken from Brownsey & Smith-Dodsworth 1989, Spore pictures from Large & Braggins 1991.

In summary, the *trnL-trnF* tree produced in this study appears well supported, with agreement between several different molecular methods and morphological data.

In the *rbcL* trees presented in Section 3.1, *Sphaeropteris medullaris* appears basal to genus, as in Conant *et al.* (1994). *Sphaeropteris medullaris* has a very large distribution, throughout New Zealand and the Pacific Islands, and probably represents an early divergence of the *Sphaeropteris* lineage.

Antiquity of the NZ Cyatheaceae

Levels of sequence variation among the genera *Alsophila*, *Sphaeropteris* and *Dicksonia* are similar in both *rbcL* and the *trnL-trnF* spacer. However, the *Dicksonia* sequences in this study are much more representative of the genus than those of *Alsophila* or *Sphaeropteris*. The Cyatheaceae genera are much both much larger than *Dicksonia*, and less sequences have been included in this study. This indicates that the variability among them has been significantly underestimated. The *18S* sequences, while covering relatively few species, show greater variation than *Dicksonia*. This would suggest that speciation within *Alsophila* and *Sphaeropteris* occurred earlier than in *Dicksonia*.

Questions of the origins of these species within New Zealand, and their dispersal to or from New Zealand were unable to be addressed by this research, due to a lack of time and available materials. Further research, with representative species of *Alsophila* and *Sphaeropteris* from Australia, South America and the Pacific is required to address such questions.

5.2.2 *Hymenophyllopsis*

The *rbcL* data presented in this thesis (Figures 3.1, 3.2) provides evidence that *Hymenophyllopsis* falls within the Cyatheaceae. Three lineages appear in the *rbcL* tree, representing *Alsophila*, *Sphaeropteris* and *Hymenophyllopsis*. The *Hymenophyllopsis* branch appears to have diverged at approximately the same time as the *Alsophila* and *Sphaeropteris* lines. Unfortunately, no sequences or tissue samples from the *Cyathea* clade were available at the time this thesis was written. The *Cyathea delgadii* sample used in this study proved to be mislabelled (actually a *Sphaeropteris*), and some available sequences labelled as *Cyathea* on GenBank are also of *Sphaeropteris* (reflecting older classifications of the Cyatheaceae). Without this data it

is difficult to tell whether *Hymenophyllopsis* represents a fourth lineage within the Cyatheaceae, or whether it would lie somewhere within the *Cyathea* clade. This new placement of *Hymenophyllopsis* is very interesting given the relative uniformity of the large Cyatheaceae family.

Species of *Hymenophyllopsis* are relatively small, and have some morphological characters which superficially resemble those of *Hymenophyllum*, such as thin leaves lacking stomata. Other characters, such as scale and spore morphology, support relationships with the Cyatheaceae. These latter characters do tend to be more highly conserved (Smith 1995), and add support for the inclusion of *Hymenophyllopsis* within the Cyatheaceae. Although the possibility exists that *Hymenophllopsis* has acquired its *rbcL* gene from some chloroplast capture event, artificially placing it within the Cyatheaceae, this seems highly unlikely, given the conserved morphological characters supporting it's inclusion in the family. Further study is required to rule out this possibility, e.g. a chromosome count, *18S* sequencing. Unfortunately samples of this fern are difficult to obtain, and no sample was available for this study.

In Conant *et al.* (1996) a striking congruence between spore morphology and the evolutionary lineages observed from chloroplast restriction site data was observed. For example, echinate spores were observed only in the *Sphaeropteris* clade, and ridged spores only in *Alsophila*. Even within the *Cyathea* clade, spore morphology was unique to sub-lineages, e.g *Cnemidaria* with triporate spores, and the *Cyathea divergens* group with verrucate spores. *Hymenophyllopsis* also possess verrucate spores with surface rodlets (see Tryon & Lugardon 1991, figures 81.1 – 81.4) almost identical to those observed in the *Cyathea divergens* group. The likelihood of this unique spore type appearing more than once seems very low, suggesting that *Hymenophyllopsis* may be a subgroup within *Cyathea*. Based on spore evidence, it could be proposed that *Hymenophyllopsis* is a member of Conant's *Cyathea* clade, and may be closely related to members of the *Cyathea divergens* group. It has been noted, e.g. Tryon & Tryon (1982), that the *Cyathea divergens* complex (characterised by sphaeropteroid indusia) presents difficult taxonomic problems, containing as it does several critical and highly variable species.

If this is correct, *Hymenophyllopsis* must have evolved several morphological characters unique among the Cyatheaceae in response to the specific environment of the Mt. Roraima sandstone formation in Guyana, including a large reduction in size, possibly due to limited nutrient availability. It has been noted that the spores are large, up to twice the size of those of *Cyathea*, indicating polyploidy may have occurred, and thus providing a mechanism for radical morphological change. This would require further study to confirm, as it has been observed (Large & Braggins 1990) that different treatments can radically affect the size of spores.

Summary and Conclusions

Studies on the New Zealand tree ferns performed in this thesis represent the first molecular study of these plants. DNA sequencing has been performed on all representatives of this group, as well as on available relatives from overseas. Three molecular markers were successfully utilized in this study, the chloroplast markers *rbcL* and the *trnL-trnF* spacer, and the nuclear *18S* rDNA. The non-coding *trnL-trnF* region provided the most variable marker, useful for studying lower level phylogenetic relationships. The *rbcL* gene is slower evolving, and has a wide range of previously sequenced taxa available on GenBank, and was able to provide an overview of relationships among the tree fern clade. The *18S* rDNA was found to possess little variability, and as such, may be useful for future studies investigating the origins of the tree fern clade.

The *rbcL* studies on the tree fern clade provided a strong indication of relationships among the ingroup taxa: the families Cyatheaceae, Dicksoniaceae, Hymenophyllopsidaceae and Lophosoriaceae. These appear to fall into three major lineages: The genera *Calochlaena*, *Culcita*, *Dicksonia* and *Lophosoria* form the first; *Cibotium* the second; and the Cyatheaceae and Hymenophyllopsidaceae the third. However, relationships between these and outgroup taxa (*Azolla*, *Loxsoma*, *Metaxya* and *Plagiogyria*) were not so clearly resolved, probably due to multiple substitutions at variable sites. For this reason, the rooted phylogeny given in Figure 3.2 is tentative.

Studies on the genus *Dicksonia* using the chloroplast markers identified evidence of three distinct genetic lineages that correlate with three different spore morphologies: smooth, tuberculate and intermediate. The New Zealand species fall into two of these lineages: *Dicksonia fibrosa* in the smooth spore group, and *D. lanata* and *D. squarrosa* in the tuberculate spore group. In addition, the three lineages appear to have diverged at approximately the same time, most likely late in the Tertiary (65 – 2 million years ago). Although the smooth spore group (represented here by *D. antarctica*, *D. fibrosa* and *D. sellowiana*) may be an old lineage (fossils know from the Jurassic, e.g. Van Konjinenberg-Van Cittert 1989), the species appear very recent, possibly as recent as the Quartenary (the last 2 million years). The tuberculate spore

group (represented here by *D. baudouinii*, *D. lanata* and *D. squarrosa*) appear to have diverged somewhat earlier than this. However, many species remain unsequenced so speculation on the age of this group would be premature. Further study on these ferns using the *trnL-trnF* spacer may provide insights into directions of dispersal and better estimates of age.

Amplified Fragment Length Polymorphism (AFLP) studies on populations of *Dicksonia lanata* identified evidence for a genetic differentiation between two observed growth forms. Split decomposition and tree-building analysis identified a well supported split between the two population groups. This split appears to be of insufficient size to warrant recognition of the two growth forms as separate species. However, recognition at sub-species level may be appropriate. In which case, the name *Dicksonia lanata* subsp. *hispida* is available for the northern form, after original description by Colenso in 1845, the southern prostrate form becoming *Dicksonia lanata* subsp. *lanata*.

Results from studies on the New Zealand Cyatheaceae provide support for two distinct lineages, *Alsophila* and *Sphaeropteris*. This is in agreement with previous observations based on morphological data. An interesting finding of the *rbcL* data is that the South American fern genus *Hymenophyllopsis* appears to be firmly nested within the Cyatheaceae, probably most closely related to the genus *Cyathea*. Previous placements of this genus based on morphology have been highly uncertain.

The New Zealand Flora

The New Zealand flora and fauna has long been considered to have survived the break up of the Gondwana super-continent and remained largely unchanged since that time (e.g. Cockayne 1927). The tree ferns are an ancient group often associated with the Gondwanan flora. Current understanding now suggests long-distance dispersal being responsible for the current character of New Zealand's flora. Authors such as Pole (1994) and Macphail (1997) argue that New Zealand's current flora is almost entirely a result of long-distance dispersal. Studies of individual plant groups using molecular methods are increasingly adding support to the importance of this dispersal in the

New Zealand flora (e.g. Winkworth *et al.* 2000) at least with regard to the more derived plant groups. Ferns are not so well studied.

In this study, two lineages of *Dicksonia* have been found within New Zealand, having diverged probably between 9 and 65 million years ago (but closer to 9). The first is represented by *D. fibrosa*, and the second by *D. lanata/D. squarrosa*. The existence of two lineages, of relatively recent divergence, argues against a long period of isolation, and seems to fit into a general pattern within the New Zealand flora of long-distance dispersal. Close relationships (e.g. between *D. fibrosa* and *D. sellowiana* of South America) suggest the species themselves may have only evolved in the Quartenary (the last 1.6 million years), although directions of dispersal remain uncertain.

The antiquity of the Cyatheaceae species in this study are more uncertain, as the group is much larger and is poorly represented here. However, it appears from *trnL-trnF* data (which does appear to be evolving in a clock-like fashion) that either evolution of the spacer is faster in this group, or that species radiation has occurred earlier than in *Dicksonia*. Questions of the origins of these species within New Zealand, and their dispersal to or from New Zealand were unable to be addressed by this research, due to a lack of time and available materials. Further research, with representative species of *Alsophila* and *Sphaeropteris* from Australia, South America and the Pacific is required to address such questions.

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APPENDICES

APPENDIX 1

1.1 Geological Time Scale

Era	Period	Epoch
CENOZOIC (65)	Quartenary (2)	Recent (0.01)
		Pleistocene (2)
	Tertiary (65)	Pliocene (5.1)
		Miocene (24.6)
		Oligocene (38)
		Eocene (54.9)
		Paleocene (65)
MESOZOIC (248)	Cretaceous (144)	
	Jurassic (213)	
	Triassic (248)	
PALEOZOIC (590)	Permian (286)	
	Carboniferous (360)	
	Devonian (408)	
	Silurian (438)	
	Ordovician (505)	
	Cambrian (590)	

PRECAMBRIAN (4500)

Numbers in brackets indicate millions of years before present.

1.2 Authorities

Cyatheaceae Kaulfuss
Sphaeropteris Bernhardi
Alsophila R. Brown
Nephelea Tryon
Trichipteris Presl
Cyathea J. Smith
Cnemidaria Presl

Dicksoniaceae Bower
Dicksonia L'Heritier de Brutelle
Thyrsopteris Kunze

Cystodium Hooker
Cibotium Kaulfuss
Calochlaena (Maxon) Turner & White
Culcita Presl

Hymenophyllopsidaceae Pichi-Sermolli
Hymenophyllopsis Goebel
H. hymenophylloides Gomez

Lophosoriaceae Pichi-Sermolli
Lophosoria Presl
L. quadripinnata (Gemlin) C. Christensen

Loxsomataceae Presl
Loxsoma Cunningham
Loxsomopsis Christ

Metaxyaceae Pichi-Sermolli
Metaxya Presl
M. rostrata (Kunth) Presl

Plagiogyriaceae Bower
Plagiogyria (Kunze) Mettenius

1.2.1 Fossil Taxa

Alsophilocaulis Menendez
Cibotiocaulis Ogura
Coniopteris Brongniart
Conantiopteris Lantz, Rothwell & Stockey
Cyatheocaulis Ogura
Erboracia H. H. Thomas
Lophosoriorhachis Nishida
Nishidacaulis Tidwell & Nishida
Oguracaulis Tidwell *et al.*
Onychiopsis see Tidwell & Ash 1994

APPENDIX 2

2.1 Samples/Herbarium Vouchers

Sample	Extraction Code	Collector(s)	Locality	Date
Dicksonia				
D. fibrosa	FIB ROT	M.F.Large	cultivation *ex.* Rotorua	02/00
D. squarrosa	SQU SHADE	R.L.Lewis	Massey University, Palmerston North	03/00
D. antarctica	ANT TAS	P.J.Lockhart	Burraga Swamp, Barrington Mountains, Australia	06/00
D. antarctica	ANT NSW	RGB Sydney	NSW, Australia	02/00
D. antarctica	ANT VIC	RBG Sydney	Blue Mountains, Victoria, Australia	02/00
D. sellowiana	SEL SA	RGB Sydney	Chile	02/00
D. berteriana	BER 1	C.Lehenbeck	Chile	01/01
D. baudouinii	BAU 1	E.Cameron	New Caledonia	02/00
D. arborescens	ARB 1	Q.B.Cronk	Edinburgh BG *ex.* St. Helena	99
Alsophila				
A. tricolor	TRI 1	R.L.Lewis/M.F.Large	Ohakune	03/00
A. cunninghamii	CUN 1	Auckland University	cultivation	03/00
A. smithii	SMI 1	M.F.Large	Tongariro	03/00
A. kermadecensis	KER 1	Auckland University	cultivation *ex.* Raoul Island	03/00
A. milnei	MIL 1	Auckland University	cultivation *ex.* Raoul Island	03/00
A. colensoi	COL 1	R.L.Lewis/M.F.Large	Tongariro	03/00
Sphaeropteris				
S. medullaris	MED TECH	R.L.Lewis	Massey University, Palmerston North	03/00
S. excelsa	BRO 1	RBG Sydney	cultivation *ex.* Lord Howe Island	03/00
S. robusta	ROB 1	RBG Sydney	cultivation *ex.* Norfolk Island	03/00
S. tomentosissima	TOM 1	S.Van der Mast	Trigg Road Fern Nursery	04/00
Cyathea				
C. delgadii	DEL 1	S.Van der Mast	Trigg Road Fern Nursery	04/00
Lophosoria				
L. quadripinnata	LOPH 1	B.S.Parris	Mexico	06/00
Cibotium				
C. glaucum	CIB 1	B.S.Parris	Hawaii	06/00

2.1.1 *Dicksonia lanata Herbarium Vouchers*

	Population		*Voucher*	Region	Locality	Collector	Date	NZMS 260 Ref	Latitude	Longitude	Altitude	Notes
Northern												
	Omahuta		*Perrie DicLan Oma1*	Northland	Omahuta Kauri Sanctuary (western side of Puketi Forest)	L.R. Perrie & L.D. Shepherd	Jan 2000	O05 680620	S 36° 15'	E 173° 38'	340m	*Agathis australis / Ixerba brexioides / Dicksonia lanata & Astelia trinervia & Gahnia sp.* . Trunk c. 50cm tall.
	Omahuta		*Perrie DicLan Oma2*	Northland	Omahuta Kauri Sanctuary (western side of Puketi Forest)	L.R. Perrie & L.D. Shepherd	Jan 2000	O05 680620	S 36° 15'	E 173° 38'	340m	*Agathis australis / Weinmannia silvicola / Dicksonia lanata & D. squarrosa*. Trunk c. 70cm tall.
	Omahuta		*Perrie DicLan Oma3*	Northland	Omahuta Kauri Sanctuary (western side of Puketi Forest)	L.R. Perrie & L.D. Shepherd	Jan 2000	O05 680620	S 36° 15'	E 173° 38'	340m	*Agathis australis / Weinmannia silvicola / Dicksonia lanata & D. squarrosa*. Trunk c. 70cm tall.
	Puketi		*Perrie DicLan Puk1*	Northland	Puketi Forest Nature Trail (eastern side of Puketi Forest)	L.R. Perrie & L.D. Shepherd	Jan 2000	P05 830654	S 35° 13'	E 173° 47'	300m	*Podocarpus hallii* & *Weinmannia silvicola / Cyathea dealbata* & *Melicytus macrophyllus*. Trunk c. 1m tall. Little grove
	Puketi		*Perrie DicLan Puk2*	Northland	Puketi Forest Nature Trail (eastern side of Puketi Forest)	L.R. Perrie & L.D. Shepherd	Jan 2000	P05 830654	S 35° 13'	E 173° 47'	300m	*Weinmannia silvicola* & *Knightia excelsa* & *Olearia rani / Cyathea dealbata*. Trunk c. 50cm tall. In small clearing, in full sunlight.
	Puketi		*Perrie DicLan Puk3*	Northland	Puketi Forest Nature Trail (eastern side of Puketi Forest)	L.R. Perrie & L.D. Shepherd	Jan 2000	P05 830654	S 35° 13'	E 173° 47'	300m	About 10m from *Perrie DicLan Puk2*, in shade of *Weinmannia silvicola* & *Cyathodes sp.* Truck c. 80cm tall, quite thick.

	Russell		*Perrie DicLan Rus1*	Northland	In Russell Forest, from Punaruku Road near Oakura.	L.R. Perrie & L.D. Shepherd	Jan 2000	Q05 280432	S 35° 24'	E 174° 17'	100m	Hillside. *Kunzea ericoides & Phyllocladus trichomanoides / Podocarpus hallii* (saplings) & *Leucopogon fasciculatus*. Very dense growth of *D. lanata*. No evidence of trunks, but many plants fertile. Fronds up to c. 1.5m long.
	Russell		*Perrie DicLan Rus2*	Northland	In Russell Forest, from Punaruku Road near Oakura.	L.R. Perrie & L.D. Shepherd	Jan 2000	Q05 280432	S 35° 24'	E 174° 17'	100m	Hillside. *Kunzea ericoides & Phyllocladus trichomanoides / Podocarpus hallii* (saplings) & *Leucopogon fasciculatus*. Very dense growth of *D. lanata*. No evidence of trunks, but many plants fertile. Fronds up to c. 1.5m long.
	Trounson		*Perrie DicLan Tro1*	Northland	Trounson Forest	L.R. Perrie & L.D. Shepherd	Jan 2000	O07 695088	S 35° 44'	E 173° 39'	240m	Trunk c. 50cm tall. *Agathis australis / Beilschmiedia tawa & B. tarairi & Weinmannia silvicola // D. lanata.*
	Trounson		*Perrie DicLan Tro2*	Northland	Trounson Forest	L.R. Perrie & L.D. Shepherd	Jan 2000	O07 695088	S 35° 44'	E 173° 39'	240m	Trunk c. 40cm tall. *Agathis australis / Beilschmiedia tarairi & Weinmannia silvicola // D. lanata.*
	Waipoua		*Perrie DicLan Wpu1*	Northland	Near the "Four Sisters" kauri trees in Waipoua Forest	L.R. Perrie & L.D. Shepherd	Jan 2000	O06 585213	S 35° 37'	E 173° 31'	320m	Trunk c. 70cm tall. *Agathis australis / Quintinia serrata & Olearia rani / Gahnia sp.*
	Waipoua		*Perrie DicLan Wpu2*	Northland	On Te Matua Ngahere track in Waipoua Forest	L.R. Perrie & L.D. Shepherd	Jan 2000	O06 585213	S 35° 37'	E 173° 31'	320m	Trunked. *Agathis australis / Quintinia serrata / D. lanata & Astelia trinervia & Gahnia sp.*
	Herekino		*Perrie DicLan Her1*	Northland	Western side of Herekino Forest. At top of ridge	L.R. Perrie & L.D. Shepherd	Jan 2000	N05 284658	S 35° 17'	E 173° 11'	c. 250m	Small colony. Trunk c. 60cm tall. *Dacrydium cupressinum (juv.) & Kunzea*

					on unmarked track which begins at the peak of the road between Ahipara and Herekino township.							*ericoides / Gahnia sp.*
	Herekino		*Perrie DicLan Her2*	Northland	Western side of Herekino Forest. At top of ridge on unmarked track which begins at the peak of the road between Ahipara and Herekino township.	L.R. Perrie & L.D. Shepherd	Jan 2000	N05 284658	S 35° 17'	E 173° 11'	c. 250m	Small colony. Trunk c. 60cm tall. *Dacrydium cupressinum (juv.) & Kunzea ericoides / Gahnia sp.*
	Whangarei		*Perrie DicLan Whg1*	Northland	About 5 minutes down the north-western fork of the Pukenui Forest track, near Whangarei	L.R. Perrie & L.D. Shepherd	Jan 2000	Q07 243097	S 35° 43'	E 174° 15'	200m	Small grove. Trunked. *Phyllocladus trichomanoides & Weinmannia silvicola & Beilschmiedia tarairi & Dacrydium cupressinum / Cyathea medullaris & C. dealbata & Coprosma robusta & Melicytus ramiflorus.*
Southern												
	Coromandel		*Perrie DicLan Cor1*	Coromandel	About 1h 15min along Tararua Valley track, western side of the Coromandel Ranges	L.R. Perrie & L.D. Shepherd	Jan 2000	T12 c. 40-55-	S 35° 05'	E 175° 35'	c. 600m	Appears to be creeping form. No evidence of trunk despite plants being quite big, and fertile. Stipe up to c. 1m, rachis c. 1.2m. Ridge top. Wet forest. *Weinmannia silvicola* and *Quintinia serrata* over *Dicksonia squarrosa*

	Coromandel		*Perrie DicLan Cor2*	Coromandel	About 1h 15min along Tararua Valley track, western side of the Coromandel Ranges	L.R. Perrie & L.D. Shepherd	Jan 2000	T12 c. 40-55-	S 35° 05'	E 175° 35'	c. 600m	Appears to be creeping form. No evidence of trunk despite plants being quite big, and fertile. Stipe up to c. 1m, rachis c. 1.2m. Ridge top. Wet forest. *Weinmannia silvicola* and *Quintinia serrata* over *Dicksonia squarrosa*
	Coromandel		*Perrie DicLan Cor3*	Coromandel	About 1h 15min along Tararua Valley track, western side of the Coromandel Ranges	L.R. Perrie & L.D. Shepherd	Jan 2000	T12 c. 40-55-	S 35° 05'	E 175° 35'	c. 600m	Appears to be creeping form. No evidence of trunk despite plants being quite big, and fertile. Stipe up to c. 1m, rachis c. 1.2m. Ridge top. Wet forest. *Weinmannia silvicola* and *Quintinia serrata* over *Dicksonia squarrosa*
	Hauhangatahi		*DicLan Hau1*	Ruapehu	About 30 min along Hauhangatahi Track	R.L. Lewis/M.F. Large	Apr 2000	?	?	?	?	
	Kinwig		*Perrie DicLan Rua3*	Ruahine	Ruahines. On Knight's Track, from Unutoi North Road to Leon Kinvig Hut.	L.R. Perrie & L.D. Shepherd	1999	T22 69-30-	S 39° 59'	E 176° 02'	c. 1000 m	
	Kinwig		*Perrie DicLan Rua4*	Ruahine	Ruahines. On Knight's Track, from Unutoi North Road to Leon Kinvig Hut.	L.R. Perrie & L.D. Shepherd	1999	T22 69-30-	S 39° 59'	E 176° 02'	c. 1000 m	
	Napier		*Perrie DicLan Bel1*	Napier	Near the entrance to Bellbird Bush Scenic Reserve,	L.R. Perrie & L.D. Shepherd	Jun 2000	V19 390235	S 39° 08'	E 176° 47'	c. 750m	Small colony. Scattered elsewhere. Under canopy of *Nothofagus fusca* & *Dacrydium cupressinum*, with

					which is north of Napier, near Mokara							understorey of *Pseudowintera colorata*.
	Ohakune		*DicLan Oha1*	Ruapehu	Alongside road from Ohakune to Turoa	R.L. Lewis/M.F. Large	Apr 2000	S20 22-01-	S 39° 22'	E 175° 27'	c. 800m	
	Pirongia		*Perrie DicLan Pir1*	Pirongia	Between Ruapane and Tirohanga, on eastern side of Mt. Pirongia	L.R. Perrie & L.D. Shepherd	Dec 1999	S15 96-56-	S 37° 59'	E 175° 07'	c. 720m	Small colony, sited on top of ridge. In open, exposed conditions.
	Pirongia		*Perrie DicLan Pir2*	Pirongia	Between Ruapane and Tirohanga, on eastern side of Mt. Pirongia	L.R. Perrie & L.D. Shepherd	Dec 1999	S15 96-56-	S 37° 59'	E 175° 07'	c. 720m	Small colony, sited on top of ridge. In open, exposed conditions.
	Pirongia		*Perrie DicLan Pir3*	Pirongia	Between Ruapane and Tirohanga, on eastern side of Mt. Pirongia	L.R. Perrie & L.D. Shepherd	Dec 1999	S15 96-56-	S 37° 59'	E 175° 07'	c. 720m	Small colony, sited on top of ridge. In open, exposed conditions.
	Pirongia		*Perrie DicLan Pir4*	Pirongia	Between Ruapane and Tirohanga, on eastern side of Mt. Pirongia	L.R. Perrie & L.D. Shepherd	Dec 1999	S15 96-56-	S 37° 59'	E 175° 07'	c. 720m	Small colony, sited on top of ridge. In open, exposed conditions.
	Potae		*Perrie DicLan Rua5*	Ruahine	North-western Ruahines. Near Potae on track between Lake Colenso and Ruahine Corner hut.	L.R. Perrie & L.D. Shepherd	2000	U21 81-67-	S 39° 40'	E 176° 09'	c. 900m	
	Urewera		*Perrie*	Urewera	From the	L.R. Perrie & R.W.	1998	W18 64-	S 38°	E 177°	c.	On hillside. Large colony.

			DicLan Ure1		Hopuruahine Stream catchment, north of State Highway 38.	Perrie		73-	41'	05'	750m	*Nothofagus fusca* forest.
	Rangiwahia		*Perrie DicLan Rua1*	Ruahine	On track up to Rangiwahia hut	L.R. Perrie & L.D. Shepherd	21 April 1999	T22 67-41-	S 39° 54'	E 176° 01'	c. 900m	
	Mokai		*Perrie DicLan Rua6*	Ruahine	North western Ruahines. On track from Mokai Station to Iron Bark hut.	L.R. Perrie & L.D. Shepherd	2000	U21 74-644	S 39° 41'	E 176° 05'	c. 800m	
	Mangaweka		*Perrie DicLan Rua2*	Ruahine	Track to Mt. Mangaweka summit	L.D. Shepherd	1999	U22 70-498	S 39° 49'	E 176° 03'	c. 1000 m	
	Kaweka		*Perrie DicLan Kaw1*	Kaweka Range	About 15 minutes walk south-west along track from Middle Hill hut.	L.R. Perrie & L.D. Shepherd	1999	U20 055137	S 39° 14'	E 176° 25'	c. 950m	Scattered
	Kaimanawas		*Perrie DicLan Kmw1*	Kaimana was	A couple of hours walk along track from end of Clements Hill Road.	L.R. Perrie	Dec 1999	U19 81-36-	S 39° 02'	E 176° 09'	c. 1000 m	North eastern side of ridge top. *Nothofagus menziesii* and *Pseudowintera colorata*.

2.2 DNA Extraction

CTAB solution (40mL)

0.8g CTAB (2% w/v)
0.4g PVP (1% w/v)
11.2mL 5M NaCl (1.4M)
8mL 100mM EDTA (20mM)
4mL Tris HCl pH8 (100mM)
make up to 40mL with MilliQ H_2O

TE buffer (10:1 Tris EDTA buffer)

10 mM Tris-HCl
1 mM EDTA (pH 8.0)

TAE buffer (1× Tris acetate EDTA buffer)

40 mM Tris acetate
1 mM EDTA pH 8.0

1% (w/v) Agarose Gel

1 g Seakem LE agarose (FMC BioProducts)
100 mL 1× TAE buffer

Loading Buffer

27.5% (w/v) Ficoll Ty 400 (Pharmarcia)
0.44% (w/v) bromophenol blue (Serva)
0.44% (w/v) xylene cyanol (Sigma)

2.3 PCR & Sequencing

2.3.1 Primer Sequences (5' - 3')

All primers either from Sigma or Gibco BRL.

TabC	CGAAATCGGTAGACGCTACG
TabD	GGGGATAGAGGGACTTGAAC
TabE	GGTTCAAGTCCCTCTATCCC
TabF	ATTTGAACTGGTGACACCAG
18sF	AACCTGGTTGATCCTGCCAGT
18sR	GATCCTTCTGCAGGTTCACCTAC
18sF2	GTAGTTGGATCTCGGGGCG
18sR2	CCGTCAATTCCTTTAAGTTTC

aF	ATGTCACCACAAACAGAGACTAAAGC
cR	GCAGCAGCTAGTTCCGGGCTCCA
422F	GCTTGGAAGACCTTCGAATTC
961F	GTATTGGCCAAAGCATTACGCATG
579R	GTGAAATCAAGTCCGCCGCG
988R	CCTCCAGTTTACCTACTACAG
579Rdic	GTGAAGTCAAGTCCACCACG
ITS4	TCCTCCGCTTATTGATATGC
ITS5	GGAAGTAAAAGTCGTAACAAGG
ITS2	GCTACGTTCTTCATCGATGC
rps4F	ATGTCCCGTTATCGAGGAACCT
rps2	GTACCACTGCAATTACTC
trnS	TACCGAGGGTTCGAATC

2.3.2 PCR Reaction cocktail

9 µL MilliQ H_2O
4 µL Q solution (Qiagen)
2 µL 10X PCR buffer (Qiagen)
2.5 µL dNTP's (250 µmol, Boehringer Mannheim)
1 µL Primer 1 (10 pmol)
1 µL Primer 2 (10 pmol)
0.2 µL Taq polymerase (Qiagen or Gibco BRL)
total volume 19.7 µL

2.3.3 PCR Programs

AUTOSEQ

1: 96 °C for 10 seconds (denaturation)
2: 50 °C for 5 seconds (annealing)
3: 60 °C for 4 minutes (extension)
4: 25 times to 1
5: 4 °C for 12 minutes
6: end

TABER

1: 94 °C for 2 minutes
2: 94 °C for 1 minute (denaturation)
3: 53 °C for 1 minute (annealing)
4: 72 °C for 1 minute (extension)
5: 40 times to 2
6: 72 °C for 7 minutes
7: 4 °C for ever

8: end

ITS

1: 94 °C for 2 minutes
2: 94 °C for 30 seconds (denaturation)
3: 50 °C for 30 seconds (annealing)
4: 72 °C for 1 minute (extension)
5: 34 times to 2
6: 72 °C for 5 minutes
7: 4 °C for ever
8: end

RPS4

1: 94 °C for 2 minutes
2: 94 °C for 1 minute (denaturation)
3: 50 °C for 1 minute (annealing)
4: 72 °C for 45 seconds (extension)
5: 37 times to 2
6: 72 °C for 5 minutes
7: 4 °C for ever
8: end

2.3.4 Equipment

PCR Machines

PTC-200 DNA Engine (MJ Research)
PTC-150 Minicycler (MJ Research)

Automated Sequencer

373A DNA Sequencing System (Applied Biosystems)

2.4 AFLP

2.4.1 Primer Sequences

Pre-amplification

Eco Preamp – A	GACTGCGTACCAATTCA
Mse Preamp – C	GATGAGTCCTGAGTAAC

Selective Amplification

Eco-AAT	GACTGCGTACCAATTCAAT
Eco-ATA	GACTGCGTACCAATTCATA
Eco-AGC	GACTGCGTACCAATTCAGC
Mse-CTG	GATGAGTCCTGAGTAACTG
Mse-CAG	GATGAGTCCTGAGTAACAG

2.4.2 PCR Reactions

AFLP Pre-amplification

20.7 µL total volume
9 or 10 µL MilliQ H_2O (depending on whether 1 or 2 primers were used)
4 µL Q solution (Qiagen)
2 µL 10X PCR reaction buffer (Qiagen)
2.5 µL dNTP's (250 µmol, Boehringer Mannheim)
1 µL Eco+N primer and/or 1µL Mse+N primer (10 pmol)
0.2 µL Taq polymerase (Qiagen or Gibco BRL)

AFLP Selective Amplification

19.65 µL total volume
10.5-11.5 µL MilliQ H_2O (for 1 or 2 primers)
2 µL 10X PCR reaction buffer (Qiagen)
2.5 µL $MgCl_2$ solution (Qiagen)
2.5 µL dNTP's (250 µmol, Boehringer Mannheim)
1 µL Eco+ANN primer and/or 1µL MseI+CNN
0.15 µL Taq polymerase (Qiagen or Gibco BRL)

2.4.3 PCR Programs

AFLPPA

1: 1 °C/second to 94 °C
2: 94 °C for 30 seconds (denaturation)
3: 1 °C/second to 56 °C

4: 56 °C for 1 minute (annealing)
5: 1 °C/second to 72 °C
6: 72 °C for 1 minute (extension)
7: 19 times to 1
8: 4 °C for ever
9: end

AFLPSO

1: 94 °C for 2 minutes
2: 94 °C for 30 seconds (denaturation)
3: 65 °C for 30 seconds (annealing)
4: 72 °C for 1 minute (extension)
5: 5 times to 2
6: 94 °C for 30 seconds (denaturation)
7: 60 °C for 30 seconds (annealing)
8: 72 °C for 1 minute (extension)
9: 5 times to 6
10: 94 °C for 30 seconds (denaturation)
11: 56 °C for 30 seconds (annealing)
12: 72 °C for 1 minute (extension)
13: 23 times to 10
14: 72 °C for 5 minutes
15: 4 °C for ever
16: end

TBE Buffer (1x Tris borate EDTA buffer)

90mM Tris-borate
1mM EDTA pH 8.0

APPENDIX 3

3.1 *rbcL* DNA sequence alignment

Sequence Label	Species	Source/GenBank Accession
Dicksonia_antarctica_Hasebe	Dicksonia antarctica	U05618 (Hasebe *et al.* 1994)
Dicksonia_antarctica_Wolf	Dicksonia antarctica	U05919 (Wolf *et al.* 1994)
Dic_antarctica_Vic	Dicksonia antarctica	Australia/RBG Sydney
Dic_fibrosa	Dicksonia fibrosa	New Zealand/L.R. Perrie
Dic_sellowiana_SA	Dicksonia sellowiana	Chile/RBG Sydney
Dic_sellowiana_Chile	Dicksonia sellowiana	Chile/C. Lehenbeck
Dic_arborescens	Dicksonia arborescens	St. Helena/Q. Cronk
Dic_baudouinii	Dicksonia baudouinii	New Caledonia/E. Cameron
Dic_squarrosa	Dicksonia squarrosa	L.R.Perrie
Dic_lanata	Dicksonia lanata	L.R.Perrie
Lophosoria_quadripinnata	Lophosoria quadripinnata	Mexico/B.S. Parris
Calochlaena_villosa	Calochlaena villosa	U05912 (Wolf *et al.* 1994)
Culcita_dubia	Culcita dubia	U05615 (Hasebe *et al.* 1994)
Culcita_conniifolia	Culcita conniifolia	U18648 (Wolf *et al.* 1994)
Cibotium_glaucum	Cibotium glaucum	U05913 (Wolf *et al.* 1994)
Cibotium_barometz	Cibotium barometz	U05610 (Hasebe *et al.* 1994)
Sphaeropteris	Sphaeropteris sp.	U05914 (Wolf *et al.* 1994)
Sphaeropteris_lepifera	Sphaeropteris lepifera	U05616 (Hasebe *et al.* 1994)
Sphaeropteris_cooperi	Sphaeropteris cooperi	U05944 (Wolf *et al.* 1994)
Sph_excelsa	Sphaeropteris excelsa	Australia/RGB Sydney
Sph_medullaris	Sphaeropteris medullaris	New Zealand/R.L. Lewis
Als_tricolor	Alsophila tricolor	New Zealand/R.L. Lewis
Als_smithii	Alsophila smithii	New Zealand/M.F. Large
Hymenophyllopsis_hymenophylloi	Hymenophyllopsis hymenophylloides	AF101302 (Wolf *et al.* 1999)
Hymenophyllopsis_dejecta	Hymenophyllopsis dejecta	AF101301 (Wolf *et al.* 1999)
Plagiogyria_japonica	Plagiogyria japonica	U05643 (Hasebe *et al.* 1994)
Loxoma_cunninghamii	Loxsoma cunninghamii	U30834 (Hasebe *et al.* 1995)
Metaxya_rostrata	Metaxya rostrata	U05635 (Hasebe *et al.* 1994)
Azolla_caroliniana	Azolla caroliniana	U24185 (Hasebe *et al.* 1995)

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                                  1                                                50
Dicksonia_antarctica_Hasebe        ---------------------------------ATTGACCTATTACACTCC
Dicksonia_antarctica_Wolf         GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Dic_antarctica_Vic                GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Dic_fibrosa                       GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Dic_sellowiana_SA                 GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Dic_sellowiana_Chile              GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Dic_arborescens                   GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Dic_baudouinii                    GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Dic_squarrosa                      ----------------------------ATCGATTGACCTATTACACTCC
Dic_lanata                         -------------------------ATTATCGATTGACCTATTACACTCC
Lophosoria_quadripinnata          GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Calochlaena_villosa                ---------------------------------ATTGACCTATTACACTCC
Culcita_dubia                      ------------GCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Culcita_conniifolia                ------------GTTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Cibotium_glaucum                  GTTGGATTCAAAGCTGGTGTTAAAGATTATCGGTTGACCTATTACACTCC
Cibotium_barometz                  ----------------------------------GACCTATTACACTCC
Sphaeropteris                     GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Sphaeropteris_lepifera             ----------------------AGATTATCGATTGACCTATTACACTCC
Sphaeropteris_cooperi             GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Sph_excelsa                       GTTGGATTCAAAGCTCGTGTTAAAGATTATCGATTGACCTATTACACTCC
Sph_medullaris                    GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Als_tricolor                      GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Als_smithii                       GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Hymenophyllopsis_hymenophylloi    GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
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Hymenophyllopsis_dejecta          GTTGGATTCAAAGCTGGTGTTAAAGATTATCGATTGACCTATTACACTCC
Plagiogyria_japonica              ------------------------------TTGACCTATTACACTCC
Loxoma_cunninghamii               ----------------------------------------TACACTCC
Metaxya_rostrata                  ---------------GTGTTAAAGACTATCGATTGACCTATTACACTCC
Azolla_caroliniana                ---------------------------ATCGATTGACCTATTACACTCC

                                  51                                            100
Dicksonia_antarctica_Hasebe       CGATTATGCGACCAAAGACACCGATATCTTGGCGGCCTTTCGAATGACCC
Dicksonia_antarctica_Wolf         CGATTATCCGACCAAAGACACCGATATCTTGGCGGCCTTCCGAATGACCC
Dic_antarctica_Vic                CGATTATGCGACCAAAGACACCGATATCTTGGCGGCCTTTCGAATGACCC
Dic_fibrosa                       CGATTATGCGACCAAAGACACCGATATCTTGGCGGCCTTTCGAATGACCC
Dic_sellowiana_SA                 CGATTATGCGACCAAAGACACCGATATCTTGGCGGCCTTTCGAATGACCC
Dic_sellowiana_Chile              CGATTATGCGACCAAAGACACCGATATCTTGGCGGCCTTTCGAATGACCC
Dic_arborescens                   CGATTATACGACCAAAGACACCGATATCTTGGCGGCCTTTCGAATGACCC
Dic_baudouinii                    CGATTATCCGACCAAAGACACCGATATCTTGGCGGCCTTTCGAATGACCC
Dic_squarrosa                     CGATTATCCGACCAAAGACACCGATATCTTGGCGGCCTTTCGAATGACCC
Dic_lanata                        CGATTATCCGACCAAAGACACCGATATCTTGGCGGCCTTTCGAATGACCC
Lophosoria_quadripinnata          CGATTATGCGACCAAAGACACCGATATCTTGGCGGCCTTTCGAATGACCC
Calochlaena_villosa               CGATTATGTAACCAAAGACACCGATATATTGGCGGCCTTTCGAATGACCC
Culcita_dubia                     CGATTATGTAACCAAAGACACCGATATATTGGCGGCCTTTCGAATGACCC
Culcita_conniifolia               CGATTATGTAACCAAAGACACCGATATATTGGCGGCCTTTCGAATGACCC
Cibotium_glaucum                  CGATTATAAGACCAAAGACACCGATATCTTGGCAGCCTTTCGAATCACCC
Cibotium_barometz                 CGATTATGAGACCAAAGACACCGATATCTTGGCAGCCTTTCGAGTAACCC
Sphaeropteris                     CAAGTATGAGACCAAAGACACCGATATCTTGGCAGCCTTTCGAATGACCC
Sphaeropteris_lepifera            CAAGTATGAGACCAAAGACACCGATATCTTGGCAGCCTTTCGAATGACCC
Sphaeropteris_cooperi             CAAGTATGAGACCAAAGACACCGATATCTTGGCAGCCTTTCGAATGACCC
Sph_excelsa                       CAAGTATGAGACCAAAGACACCGATATCTTGGCAGCCTTTCGAATGACCC
Sph_medullaris                    CAAGTATGAGACCAAAGACACCGATATCTTGGCAGCCTTTCGAATGACCC
Als_tricolor                      CAAGTATGAGACCAAAGACACCGATATCTTGGCAGCCTTTCGAATGACCC
Als_smithii                       CAAGTATGAGACCAAAGACACCGATATCTTGGCAGCCTTTCGAATGACCC
Hymenophyllopsis_hymenophylloi    CAAGTATGAGACCAAAGACACCGATATATTGGCAGCCTTTCGAATGACCC
Hymenophyllopsis_dejecta          CAAGTATGAGACCAAAGACACCGATATCTTGGCAGCCTTTCGAATGACCC
Plagiogyria_japonica              CGATTATGCGACCAAAGACACTGATATCTCGGCAGCTTTCGGAATGACCC
Loxoma_cunninghamii               CGATTATACGACCAAAGATACTGATATCTCGGCAGCCTTTCGAATGACCC
Metaxya_rostrata                  CGATTACGTAACCAAAGACACCGATATCTTGGCGGCCTTTCGGATGACCC
Azolla_caroliniana                CGATTATGTTACCAAAGATACCGATATTTTGGCAGCTTTCCGAATGACCC

                                  101                                           150
Dicksonia_antarctica_Hasebe       CGCAACCCGGAGTACCGCCTGAGGGAGCTGGAGCTGCAGTAGCTGCGGAA
Dicksonia_antarctica_Wolf         CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Dic_antarctica_Vic                CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Dic_fibrosa                       CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Dic_sellowiana_SA                 CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Dic_sellowiana_Chile              CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Dic_arborescens                   CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Dic_baudouinii                    CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCGGTAGCTGCGGAA
Dic_squarrosa                     CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCGGTAGCTGCGGAA
Dic_lanata                        CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCGGTAGCTGCGGAA
Lophosoria_quadripinnata          CGCAACCCGGAGTACCGGCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Calochlaena_villosa               CGCAACCCGGAGTACCGCCCGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Culcita_dubia                     CGCAACCCGGAGTACCGCCCGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Culcita_conniifolia               CGCAACCCGGAGTACCGCCCGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Cibotium_glaucum                  CGCAACCCGGAGTACCAGCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Cibotium_barometz                 CGCAACCCGGAGTTCCGCCTGAGGGAGCAGGGGCTGCAGTAGCTGCGGAA
Sphaeropteris                     CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Sphaeropteris_lepifera            CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Sphaeropteris_cooperi             CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Sph_excelsa                       CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Sph_medullaris                    CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Als_tricolor                      CGCAACCCGGAGTACCAGCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Als_smithii                       CGCAACCCGGAGTACCAGCTGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Hymenophyllopsis_hymenophylloi    CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTGGCTGCGGAA
Hymenophyllopsis_dejecta          CGCAACCCGGAGTACCGCCTGAGGAAGCTGGAGCTGCAGTGGCTGCGGAA
Plagiogyria_japonica              CGCAACCCGGAGTGCCACCTGAGGAAGCTGGAGCTGCAGTAGCCGCGGAA
Loxoma_cunninghamii               CGCAACCCGGAGTACCGCCCGAGGAAGCTGGAGCTGCAGTAGCTGCGGAA
Metaxya_rostrata                  CGCAACCCGGAGTACCGGCTGAGGAAGCCGGAGCCGCGGTAGCAGCGGAA
Azolla_caroliniana                CGCAACCCGGAGTCCCACCCGAAGAGGCTGGAGCTGCGGTAGCTGCGGAA

                                  151                                           200
Dicksonia_antarctica_Hasebe       TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Dicksonia_antarctica_Wolf         TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Dic_antarctica_Vic                TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Dic_fibrosa                       TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Dic_sellowiana_SA                 TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Dic_sellowiana_Chile              TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Dic_arborescens                   TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Dic_baudouinii                    TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Dic_squarrosa                     TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Dic_lanata                        TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Lophosoria_quadripinnata          TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Calochlaena_villosa               TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Culcita_dubia                     TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Culcita_conniifolia               TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Cibotium_glaucum                  TCTTCCACAGGTACATGGACCACTGTTATGACAGATGGATTTTCTAGTCT
Cibotium_barometz                 TCTTCCACAGGTACATGGACCACTGTATGGACCGACGGGCTTACCAGTCT
Sphaeropteris                     TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Sphaeropteris_lepifera            TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
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Sphaeropteris_cooperi                TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Sph_excelsa                          TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Sph_medullaris                       TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Als_tricolor                         TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Als_smithii                          TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Hymenophyllopsis_hymenophylloi       TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Hymenophyllopsis_dejecta             TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Plagiogyria_japonica                 TCTTCCACGGGTACATGGACCACTGTATGGACGGATGGACTTACTAGTCT
Loxoma_cunninghamii                  TCTTCCACAGGTACATGGACCACTGTATGGACGGATGGGCTTACCAGTCT
Metaxya_rostrata                     TCCTCCACGGGTACATGGACCACTGTATGGACGGATGGGCTTACCAGTCT
Azolla_caroliniana                   TCTTCTACAGGTACATGGACCACCGTATGGACAGATGGACTTACCAGTCT

                                     201                                              250
Dicksonia_antarctica_Hasebe          CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Dicksonia_antarctica_Wolf            CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Dic_antarctica_Vic                   CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Dic_fibrosa                          CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Dic_sellowiana_SA                    CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Dic_sellowiana_Chile                 CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Dic_arborescens                      CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Dic_baudouinii                       CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Dic_squarrosa                        CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Dic_lanata                           CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Lophosoria_quadripinnata             CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Calochlaena_villosa                  CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Culcita_dubia                        CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Culcita_conniifolia                  CGATCGCTACAAAGGTCGATGCTATGATATCGAACCTGTTTCTGGAGAGG
Cibotium_glaucum                     CGATCYCTTAAATGGCCGATGCTATGATATCGATCCTGTTGCTGGGGATG
Cibotium_barometz                    CGATCGCTACAAAGGCCGATGCTACCAAATCGAGCCTGTTGCTGGAGAGG
Sphaeropteris                        CGATCGCTACAAAGGCCGATGCTATGATATCGAACCTGTTGCTGGGGAGG
Sphaeropteris_lepifera               CGATCGCTACAAAGGCCGATGCTATGATATCGAACCTGTTGCTGGGGAGG
Sphaeropteris_cooperi                CGATCGCTACAAAGGCCGATGCTATGATATCGAACCTGTTGCTGGGGAGG
Sph_excelsa                          CGATCGCTACAAAGGCCGATGCTATGATATCGAACCTGTTGCTGGGGAGG
Sph_medullaris                       CGATCGCTACAAAGGCCGATGCTATGATATCGAACCTGTTGCTGGGGAGG
Als_tricolor                         CGATCGCTACAAAGGCCGATGCTATGATATCGAACCTGTTGCTGGGGAGG
Als_smithii                          CGATCGCTACAAAGGCCGATGCTATGATATCGAACCTGTTGCTGGGGAGG
Hymenophyllopsis_hymenophylloi       CGATCGCTACAAAGGCCGATGCTATGATCTCGAACCTGTTGCTGGGGAGG
Hymenophyllopsis_dejecta             CGATCGCTACAAAGGCCGATGCTATGATCTCGAACCCGTTGCTGGGGAGG
Plagiogyria_japonica                 CGATCGCTACAACGGTCGATGCTACGATATCGAACCTGTTTCTGGGGAGG
Loxoma_cunninghamii                  CGATCGATACAAAGGTCGATGCTACGATATCGAACCTGTTTCTGGGGAAG
Metaxya_rostrata                     CGATCGCTACAAAGGTCGATGCTATGATATTGAACCCGTTGCCGGAGAAG
Azolla_caroliniana                   TGACCGTTACAAAGGTAGATGCTATGATATCGAACCTGTTGCTGGAGAAG

                                     251                                              300
Dicksonia_antarctica_Hasebe          ATAATCAGTATATTGCATATGTAGCCTATCCTTTGGATCTATTTGAAGAA
Dicksonia_antarctica_Wolf            ATAAACAGTATATTGCATATCTAGCCTATCCTTTGGATCTATTTGAAGAA
Dic_antarctica_Vic                   ATAATCAGTATATTGCATATGTAGCCTATCCTTTGGATCTATTTGAAGAA
Dic_fibrosa                          ATAATCAGTATATTGCATATGTAGCCTATCCTTTGGATCTATTTGAAGAA
Dic_sellowiana_SA                    ATAATCAGTATATTGCATATGTAGCCTATCCTTTGGATCTATTTGAAGAA
Dic_sellowiana_Chile                 ATAATCAGTATATTGCATATGTAGCCTATCCTTTGGATCTATTTGAAGAA
Dic_arborescens                      ATAATCAGTATATTGCATATGTAGCCTATCCTTTGGATCTATTTGAAGAA
Dic_baudouinii                       ATAATCAGTATATTGCATATGTAGCCTATCCTTTGGATCTATTTGAAGAA
Dic_squarrosa                        ATAATCAGTATATTGCATATGTAGCCTATCCTTTGGATCTATTTGAAGAA
Dic_lanata                           ATAATCAGTATATTGCATATGTAGCCTATCCTTTGGATCTATTTGAAGAA
Lophosoria_quadripinnata             AGAATCAGTATATTGCATATGTAGCTTATCCTTTGGATCTATTTGAAGAA
Calochlaena_villosa                  AGAATCAGTATATTGCATATGTAGCTTATCCTTTGGATCTATTTGAAGAA
Culcita_dubia                        AGAATCAGTATATTGCATATGTAGCTTATCCTTTGGATCTATTTGAAGAA
Culcita_conniifolia                  AGAATCAGTATATTGCATATGTAGCTTATCCTTTGGATCTATTTGAAGAA
Cibotium_glaucum                     AGAATCAGTATATTGCATATGTAGCTTATCCTTTGGATCTATTTGAAGAA
Cibotium_barometz                    AGAATCAGTATATTGCATATGTAGCTTATCCCTTAGACCTTTTTGAAGAA
Sphaeropteris                        ATAATCAGTATATTGCATATGTAGCTTATCCTTTGGATTTATTTGAAGAA
Sphaeropteris_lepifera               ATAATCAGTATATTGCATATGTAGCTTATCCTTTGGATTTATTTGAAGAA
Sphaeropteris_cooperi                ATAATCAGTATATTGGATATGTAGCTTATCCTTTGGATTTATTTGAAGAA
Sph_excelsa                          ATAATCAGTATATTGCATATGTAGCTTATCCTTTGGATTTATTTGAAGAA
Sph_medullaris                       ATAATCAGTATATTGCATATGTAGCTTATCCTTTGGATTTATTTGAAGAA
Als_tricolor                         ATAATCAGTATATTGCATATGTAGCTTATCCTTTGGATCTATTTGAAGAA
Als_smithii                          ATAATCAGTATATTGCATATGTAGCTTATCCTTTGGATCTATTTGAAGAA
Hymenophyllopsis_hymenophylloi       ATAATCAGTATATTGCATATGTAGCTTATCCTTTGGATCTATTTGAAGAA
Hymenophyllopsis_dejecta             ATAATCAGTATATTGCATATGTAGCTTATCCTTTGGATCTATTTGAAGAA
Plagiogyria_japonica                 AAAATCAATATATTGCATATGTAGCTTACCCTCCGGATCTATTTGAAGAA
Loxoma_cunninghamii                  ATAATCAATATATCGCGTATGTAGCTTATCCTCTGGATCTATTCGAAGAA
Metaxya_rostrata                     ATAATCAGTATATTGCATATGTAGCTTATCCTTTGGATCTATTTGAAGAA
Azolla_caroliniana                   ACAACCAATACATCGCATACGTAGCTTACCCCCTAGATTTATTCGAAGAG

                                     301                                              350
Dicksonia_antarctica_Hasebe          GGTTCCGTTACCAATTTGTTCACTTCCATTGTAGGTAATGTTTTTGGATT
Dicksonia_antarctica_Wolf            GGTTCCGTTACCAATTTGTTCACTTCCATTGTAGGTAATGTTTTTGGATT
Dic_antarctica_Vic                   GGTTCCGTTACCAATTTGTTCACTTCCATTGTAGGTAATGTTTTTGGATT
Dic_fibrosa                          GGTTCCGTTACCAATTTGTTCACTTCCATTGTAGGTAATGTTTTTGGATT
Dic_sellowiana_SA                    GGTTCCGTTACCAATTTGTTCACTTCCATTGTAGGTAATGTTTTTGGATT
Dic_sellowiana_Chile                 GGTTCCGTTACCAATTTGTTCACTTCCATTGTAGGTAATGTTTTTGGATT
Dic_arborescens                      GGTTCCGTTACCAATTTGTTCACTTCCATTGTAGGTAATGTTTTTGGATT
Dic_baudouinii                       GGTTCCGTTACCAATTTGTTCACTTCCATTGTAGGTAATGTTTTTGGATT
Dic_squarrosa                        GGTTCCGTTACCAATTTGTTCACTTCCATTGTAGGTAATGTTTTTGGATT
Dic_lanata                           GGTTCCGTTACCAATTTGTTCACTTCCATTGTAGGTAATGTTTTTGGATT
Lophosoria_quadripinnata             GGTTCCGTTACCAATTTGTTCACTTCCATTGTAGGTAATGTTTTTGGATT
Calochlaena_villosa                  GGTTCCGTTACCAATATGTTCGCTTCCATTGTAGGTAATGTTTTCGGATT
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Culcita_dubia                          GGTTCCGTTACCAATATGTTCACTTCCATTGTAGGTAATGTTTTCGGATT
Culcita_conniifolia                    GGTTCCGTTACCAATATGTTCACTTCCATTGTAGGTAATGTTTTCGGATT
Cibotium_glaucum                       GGTTCCGTTACCAATKTGTTCACTTCCATTGTAGGTAATGTTTTTGGTTT
Cibotium_barometz                      GGTTCCGTTACCAACATGTTCACTTCCATTGTAGGTAATGTATTTGGGTT
Sphaeropteris                          GGTTCCGTTACCAATATGTTCACTTCCATTGTAGGTAACGTTTTTGGATT
Sphaeropteris_lepifera                 GGTTCCGTTACCAATATGTTCACTTCCATTGTAGGTAACGTTTTTGGATT
Sphaeropteris_cooperi                  GGTTCCGTTACCAATATGTTCACTTCCATCGTAGGTAACGTTTTTGGATT
Sph_excelsa                            GGTTCCGTTACCAATATGTTCACTTCCATTGTAGGTAACGTTTTTGGATT
Sph_medullaris                         GGTTCCGTTACCAACATGTTCACTTCCATCGTAGGTAACGTTTTTGGATT
Als_tricolor                           GGTTCCGTTACCAATATGTTCACTTCCATTGTAGGTAACGTTTTTGGATT
Als_smithii                            GGTTCCGTTACCAATCTGTTCACTTCCATTGTAGGTAACGTTTTTGGATT
Hymenophyllopsis_hymenophylloi         GGTTCCGTTACCAATATGTTAACTTCCATTGTAGGTAACGTTTTTGGATT
Hymenophyllopsis_dejecta               GGTTCCGTTACCAATATGTTAACTTCCATTGTAGGTAACGTTTTTGGATT
Plagiogyria_japonica                   GGGTCCGTTACCAATTCGTTCACTTCCATTGTAGGTAATGTATTTGGATT
Loxoma_cunninghamii                    GGTTCTGTTACCAATTTGTTTACTTCCATTGTAGGTAACGTTTTTGGGTT
Metaxya_rostrata                       GGTTCGGTTACCAATATGTTCACTTCCATTGTAGGTAATGTTTTTGGATT
Azolla_caroliniana                     GGTTCCGTTACCAACATGTTTACCTCCATCGTAGGTAATGTATTCGGATT

                                       351                                             400
Dicksonia_antarctica_Hasebe            CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Dicksonia_antarctica_Wolf              CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Dic_antarctica_Vic                     CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Dic_fibrosa                            CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Dic_sellowiana_SA                      CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Dic_sellowiana_Chile                   CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Dic_arborescens                        CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Dic_baudouinii                         CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Dic_squarrosa                          CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Dic_lanata                             CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Lophosoria_quadripinnata               CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Calochlaena_villosa                    CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Culcita_dubia                          CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Culcita_conniifolia                    CAAAGCCCTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Cibotium_glaucum                       CAAGGCCCTACGCGCTCTCCGTTTAGAAGATCTTCGAATTCCTCCTGCTT
Cibotium_barometz                      CAAGGCCCTACGCGCTCTACGTCTAGAGGATCTTCGAATCCCTCCTGCTT
Sphaeropteris                          CAAGGCCTTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Sphaeropteris_lepifera                 CAAGGCCTTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Sphaeropteris_cooperi                  CAAGGCCTTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Sph_excelsa                            CAAGGCCTTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Sph_medullaris                         CAAGGCCTTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Als_tricolor                           CAAGGCCTTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Als_smithii                            CAAGGCCTTACGCGCTCTCCGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Hymenophyllopsis_hymenophylloi         CAAGGCCTTACGGGCTCTACGCTTAGAAGATCTTCGGATTCCTCCTGCTT
Hymenophyllopsis_dejecta               CAAGGCCTTACGCGCTCTACGCTTAGAAGATCTTCGAATTCCTCCTGCTT
Plagiogyria_japonica                   CAAAGCCCTACGCGCTCTCCGCCTAGAAGATCCTCGAATTCCTCCTGCTT
Loxoma_cunninghamii                    CAAAGGCCTACGGGCTCTCCGCCTAGAAGATCTTCGAATTCCTCCTGCTT
Metaxya_rostrata                       CAAAGGCCTACGTGCTCTCCGCTTAGAAGATCTCCGAATTCCTCCTGCTT
Azolla_caroliniana                     TAAGGCTCTACGCGCTCTTCGCCTAGAAGATCTTCGAATTCCCCCTGCTT

                                       401                                             450
Dicksonia_antarctica_Hasebe            ATTCTAAGACTTTCATTGGACCGCCCCACGGTATCCAGGTTGAAAGGGAT
Dicksonia_antarctica_Wolf              ATTCTAAGACTTTCATTGGACCGCCCCACGGTATCCAGGTTGAAAGGGAT
Dic_antarctica_Vic                     ATTCTAAGACTTTCATTGGACCGCCCCACGGTATCCAGGTTGAAAGGGAT
Dic_fibrosa                            ATTCTAAGACTTTCATTGGACCGCCCCACGGTATCCAGGTTGAAAGGGAT
Dic_sellowiana_SA                      ATTCTAAGACTTTCATTGGACCGCCCCACGGTATCCAGGTTGAAAGGGAT
Dic_sellowiana_Chile                   ATTCTAAGACTTTCATTGGACCGCCCCACGGTATCCAGGTTGAAAGGGAT
Dic_arborescens                        ATTCTAAGACTTTCATTGGACCGCCCCACGGTATCCAGGTTGAAAGGGAT
Dic_baudouinii                         ATTCTAAGACTTTCATTGGACCGCCCCACGGTATTCAGGTTGAAAGGGAT
Dic_squarrosa                          ATTCTAAGACTTTCATTGGACCGCCCCACGGTATTCAGGTTGAAAGGGAT
Dic_lanata                             ATTCTAAGACTTTCATTGGACCGCCCCACGGTATTCAGGTTGAAAGGGAT
Lophosoria_quadripinnata               ATTCTAAGACTTTCATTGGACCGCCCCACGGTATCCAGGTTGAAAGGGAT
Calochlaena_villosa                    ATTCTAAGACTTTCATTGGACCGCCCCACGGCATCCAGGTTGAAAGAGAT
Culcita_dubia                          ATTCTAAGACTTTCATTGGACCGCCCCACGGCATCCAGGTTGAAAGAGAT
Culcita_conniifolia                    ATTCTAAGACTTTCATTGGACCGCCCCACGGCATCCAGGTTGAAAGAGAT
Cibotium_glaucum                       ATTCTAAGACTTTCATTGGCCCGCCTCACGGTATCCAGGTTGAAAGAGAT
Cibotium_barometz                      ATGCTAAGACTTTCCAAGGCCCGCCTCACGGTATCCAGGTTGAGAGAGAT
Sphaeropteris                          ATTCTAAAACTTTCATTGGACCGCCCCATGGTATCCAGGTTGAAAGGGAT
Sphaeropteris_lepifera                 ATTCTAAAACTTTCATTGGGCCGCCCCATGGTATCCAGGTTGAAAGGGAT
Sphaeropteris_cooperi                  ATTCTAAAACCTTCATTGGACCGCCCCATGGTATCCAGGTTGAAAGGGAT
Sph_excelsa                            ATTCTAAAACTTTCATTGGACCGCCCCATGGTATCCAGGTTGAAAGGGAT
Sph_medullaris                         ATTCTAAAACTTTCATTGGACCGCCCCATGGTATCCAGGTTGAAAGGGAT
Als_tricolor                           ATTCTAAAACTTTCATTGGACCGCCCCATGGTATCCAGGTTGAAAGGGAT
Als_smithii                            ATTCTAAAACTTTCATCGGACCGCCCCATGGTATCCAGGTTGAAAGGGAT
Hymenophyllopsis_hymenophylloi         ATTCTAAAACTTTCATTGGACCGCCCCATGGCATCCAGGTTGAAAGGGAT
Hymenophyllopsis_dejecta               ATTCTAAAACTTTCATTGGACCGCCCCATGGCATCCAGGTTGAAAGGGAT
Plagiogyria_japonica                   ATCCTAAGACTTTCATTGGACCGCCCCACGGTATTCAGGTCGAAAGGGAT
Loxoma_cunninghamii                    ATTCTAAGACTTTCATTGGACCTCCCCACGGTATCCAGGTCGAAAGGGAT
Metaxya_rostrata                       ATTCTAAGACTTTTATTGGACCGCCTCACGGTATCCAAGTTGAAAGAGAT
Azolla_caroliniana                     ATTCCAAAACTTTCATCGGACCACCCCACGGTATCCAGGTTGAAAGGGAC

                                       451                                             500
Dicksonia_antarctica_Hasebe            AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCAAA
Dicksonia_antarctica_Wolf              AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCAAA
Dic_antarctica_Vic                     AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCAAA
Dic_fibrosa                            AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCAAA
Dic_sellowiana_SA                      AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCAAA
Dic_sellowiana_Chile                   AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCAAA
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Dic_arborescens                    AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCAAA
Dic_baudouinii                     AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCAAA
Dic_squarrosa                      AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCAAA
Dic_lanata                         AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCAAA
Lophosoria_quadripinnata           AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCAAA
Calochlaena_villosa                AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCGAA
Culcita_dubia                      AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCGAA
Culcita_conniifolia                AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAACCGAA
Cibotium_glaucum                   AANCTGAATAAATACGGGCGTCCCTTATTAGGATGTACAATCAAGCCAAA
Cibotium_barometz                  AAGCTGAATAAATATGGGCGTCCCTTATTAGGATGTACAATCAAGCCAAA
Sphaeropteris                      AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAGCCAAA
Sphaeropteris_lepifera             AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAGCCAAA
Sphaeropteris_cooperi              AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAGCCAAA
Sph_excelsa                        AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAGCCAAA
Sph_medullaris                     AAGCTGAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAGCCAAA
Als_tricolor                       AAGCTAAACAAATATGGGCGTCCCTTATTAGGATGTACAATCAAGCCAAA
Als_smithii                        AAGCTAAACAAATATGGGCGTCCTTTATTAGGATGTACAATCAAGCCAAA
Hymenophyllopsis_hymenophylloi     AAGCTAAACAAATATGGGCGTCCCTTATTAGGGTGTACAATCAAGCCAAA
Hymenophyllopsis_dejecta           AAGCTAAACAAATATGGGCGTCCCTTATTAGGGTGTACAATCAAGCCAAA
Plagiogyria_japonica               AAACTGAACAAATACGGGCGTCCCTTATTGGGATGTACAATCAAGCCAAA
Loxoma_cunninghamii                AAACTGAACAAGTATGGGCGTCCCTTATTGGGATGTACAATCAAACCAAA
Metaxya_rostrata                   AAGCTGAACAAATATGGGCGTCCCTTCTTAGGATGTACGATTAAGCCAAA
Azolla_caroliniana                 AAACTGAACAAATATGGACGTCCTCTACTAGGATGCACGATAAAGCCAAA

                                   501                                            550
Dicksonia_antarctica_Hasebe        ATTGGGCTTATCTGCTAAGAATTATGGCAGAGCTGTTTATGAATGTCTCC
Dicksonia_antarctica_Wolf          ATTGGGCTTATCTGCTAAGAATTATGGCAGAGCTGTTTATGAATGTCTCC
Dic_antarctica_Vic                 ATTGGGCTTATCTGCTAAGAATTATGGCAGAGCTGTTTATGAATGTCTCC
Dic_fibrosa                        ATTGGGCTTATCTGCTAAGAATTATGGCAGAGCTGTTTATGAATGTCTCC
Dic_sellowiana_SA                  ATTGGGCTTATCTGCTAAGAATTATGGCAGAGCTGTTTATGAATGTCTCC
Dic_sellowiana_Chile               ATTGGGCTTATCTGCTAAGAATTATGGCAGAGCTGTTTATGAATGTCTCC
Dic_arborescens                    ATTGGGCTTATCTGCTAAGAATTATGGCAGAGCTGTTTATGAATGTCTCC
Dic_baudouinii                     ATTGGGCTTATCTGCTAAGAATTATGGCAGAGCTGTTTATGAATGTCTCC
Dic_squarrosa                      ATTGGGCTTATCTGCTAAGAATTATGGCAGAGCTGTTTATGAATGTCTCC
Dic_lanata                         ATTGGGCTTATCTGCTAAGAATTATGGCAGAGCTGTTTATGAATGTCTCC
Lophosoria_quadripinnata           ATTGGGCTTATCTGCTAAGAATTATGGGAGAGCCGTTTATGAATGTCTCC
Calochlaena_villosa                ATTGGGCTTATCTGCTAAGAATTATGGGAGAGCCGTTTATGAATGTCTCC
Culcita_dubia                      ATTGGGCTTATCTGCTAAGAATTATGGGAGAGCCGTTTATGAATGTCTCC
Culcita_conniifolia                ATTGGGCTTATCTGCTAAGAATTATGGGAGAGCCGTTTATGAATGTCTCC
Cibotium_glaucum                   ATTGGGCTTATCTGCTAAGAATTATGGGAGAGCCGTTTATGAATGTCTCC
Cibotium_barometz                  ATTGGGCTTATCTGCTAAGAATTATGGCAGAGCCGTTTATGAATGTCTCC
Sphaeropteris                      ATTGGGCTTATCTGCTAAAAATTATGGGAGAGCCGTTTATGAATGTCTCC
Sphaeropteris_lepifera             ATTGGGCTTATCTGCTAAAAATTATGGGAGAGCCGTTTATGAATGTCTCC
Sphaeropteris_cooperi              ATTGGGCTTATCTGCTAAAAATTATGGGAGAGCCGTTTATGAATGTCTCC
Sph_excelsa                        ATTGGGCTTATCTGCTAAAAATTATGGGAGAGCCGTTTATGAATGTCTCC
Sph_medullaris                     ATTGGGCTTATCTGCTAAAAATTATGGGAGAGCCGTTTATGAATGTCTCC
Als_tricolor                       ATTGGGCTTATCCGCTAAAAATTATGGGAGAGCCGTTTATGAATGTCTCC
Als_smithii                        ATTGGGCTTATCTGCTAAAAATTATGGTAGAGCCGTTTATGAATGTCTCC
Hymenophyllopsis_hymenophylloi     ATTGGGCTTATCTGCTAAGAATTATGGGAGAGCCGTTTATGAATGCCTCC
Hymenophyllopsis_dejecta           ATTGGGCTTATCTGCTAAAAATTATGGGAGAGCCGTTTATGAATGTCTCC
Plagiogyria_japonica               TTTGGGCTTATCTGCTAAGAATTATGGTAGAGCCGTTTATGAGTGTCTCC
Loxoma_cunninghamii                ATTAGGCTTATCTGCTAAGAATTATGGTAGGGCCGTTTATGAATGTCTCC
Metaxya_rostrata                   ATTGGGTTTATCCGCTAAGAACTATGGGAGAGCCGTTTATGAATGTCTCC
Azolla_caroliniana                 ATTGGGCTTATCTGCTAAGAATTATGGTAGAGCTGTTTATGAATGTCTCC

                                   551                                            600
Dicksonia_antarctica_Hasebe        GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Dicksonia_antarctica_Wolf          GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Dic_antarctica_Vic                 GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Dic_fibrosa                        GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Dic_sellowiana_SA                  GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Dic_sellowiana_Chile               GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Dic_arborescens                    GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Dic_baudouinii                     GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Dic_squarrosa                      GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Dic_lanata                         GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Lophosoria_quadripinnata           GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Calochlaena_villosa                GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCACAACCA
Culcita_dubia                      GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCACAACCA
Culcita_conniifolia                GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCACAACCA
Cibotium_glaucum                   GTGGTGGACGTGACTTCACCAAGGATGATGAGAACGTGAATTCCCAACCA
Cibotium_barometz                  GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTGAATTCCCAACCA
Sphaeropteris                      GCGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Sphaeropteris_lepifera             GCGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Sphaeropteris_cooperi              GCGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Sph_excelsa                        GCGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Sph_medullaris                     GCGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Als_tricolor                       GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Als_smithii                        GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Hymenophyllopsis_hymenophylloi     GTGGTGGACTTGATTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Hymenophyllopsis_dejecta           GTGGTGGACTTGATTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Plagiogyria_japonica               GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTCAATTCCCAACCA
Loxoma_cunninghamii                GCGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAACTTCCAACCA
Metaxya_rostrata                   GTGGTGGACTTGACTTCACCAAGGATGATGAGAACGTAAATTCCCAACCA
Azolla_caroliniana                 GCGGTGGACTTGACTTTACCAAGGATGATGAAAACGTAAACTCTCAACCA

                                   601                                            650
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Dicksonia_antarctica_Hasebe          TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Dicksonia_antarctica_Wolf            TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Dic_antarctica_Vic                   TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Dic_fibrosa                          TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Dic_sellowiana_SA                    TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Dic_sellowiana_Chile                 TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Dic_arborescens                      TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Dic_baudouinii                       TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Dic_squarrosa                        TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Dic_lanata                           TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Lophosoria_quadripinnata             TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Calochlaena_villosa                  TTCATGCGTTGGAGAGATCGTTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Culcita_dubia                        TTCATGCGTTGGAGAGATCGTTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Culcita_conniifolia                  TTCATGCGTTGGAGAGATCGTTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Cibotium_glaucum                     TTCATACGTTGGAGAGATCGCTTCTGCTTCGTAGCAGAAGCTCTCTTCAA
Cibotium_barometz                    TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Sphaeropteris                        TTCATGCGTTGGAGAGATCGTTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Sphaeropteris_lepifera               TTCATGCGTTGGAGAGATCGTTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Sphaeropteris_cooperi                TTCATGCGTTGGAGAGATCGTTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Sph_excelsa                          TTCATGCGTTGGAGAGATCGTTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Sph_medullaris                       TTCATGCGTTGGAGAGATCGTTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Als_tricolor                         TTCATGCGTTGGAGAGATCGTTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Als_smithii                          TTCATGCGTTGGAGAGATCGTTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Hymenophyllopsis_hymenophylloi       TTCATGCGTTGGAGAGATCGTTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Hymenophyllopsis_dejecta             TTCATGCGTTGGAGAGATCGTTTCTTATTCGTAGCAGAAGCTCTCTTTAA
Plagiogyria_japonica                 TTCATTCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCCCTCTTCAA
Loxoma_cunninghamii                  TTCATGCGTTGGAGAGATCGCTTCTTATTCGTAGCAGAAGCTCTCTTCAA
Metaxya_rostrata                     TTCATGCGATGGAGAGATCGCTTCTTATTTGTAGCGGAAGCTCTCTTCAA
Azolla_caroliniana                   TTCATGCGTTGGAGAGACCGTTTCCTATTCGTAGCAGAAGCTCTATTCAA

                                     651                                              700
Dicksonia_antarctica_Hasebe          AGCTCAGGCTGAAACAGGCGAAATTAAGGGACATTACCTAAATGCTACTG
Dicksonia_antarctica_Wolf            AGCTCAGGCTGAAACAGGCGAAATTAAGGGACATTACCTAAATGCTACTG
Dic_antarctica_Vic                   AGCTCAGGCTGAAACAGGCAAAATTAAGGGACATTACCTAAATGCTACTG
Dic_fibrosa                          AGCTCAGGCTGAAACAGGCGAAATTAAGGGACATTACCTAAATGCTACTG
Dic_sellowiana_SA                    AGCTCAGGCTGAAACAGGCGAAATTAAGGGACATTACCTAAATGCTACTG
Dic_sellowiana_Chile                 AGCTCAGGCTGAAACAGGCGAAATTAAGGGACATTACCTAAATGCTACTG
Dic_arborescens                      AGCTCAGGCTGAAACAGGCGAAATTAAGGGACATTACCTAAATGCTACTG
Dic_baudouinii                       AGCTCAGGCTGAAACAGGCGAAATTAAGGGACATTACCTAAATGCTACTG
Dic_squarrosa                        AGCTCAGGCTGAAACAGGCGAAATTAAGGGACATTACCTAAATGCTACTG
Dic_lanata                           AGCTCAGGCTGAAACAGGCGAAATTAAGGGACATTACCTAAATGCTACTG
Lophosoria_quadripinnata             AGCTCAGGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Calochlaena_villosa                  AGCTCAAGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Culcita_dubia                        AGCTCAAGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Culcita_conniifolia                  AGCTCAAGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Cibotium_glaucum                     AGCTCAGGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Cibotium_barometz                    AGCTCAGGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Sphaeropteris                        ATCTCAGGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Sphaeropteris_lepifera               ATCTCAGGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Sphaeropteris_cooperi                ATCTCAGGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Sph_excelsa                          ATCTCAGGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Sph_medullaris                       ATCTCAGGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Als_tricolor                         ATCTCAGGCCGAAACGGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Als_smithii                          ATCTCAGGCCGAAACGGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Hymenophyllopsis_hymenophylloi       ATCTCAGGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Hymenophyllopsis_dejecta             ATCTCAGGCCGAAACAGGCGAAATTAAGGGACATTACTTAAACGCTACTG
Plagiogyria_japonica                 AGCTCAGGCCGAAACAGGCGAAATCAAGGGACATTACTTGAATGCTACTG
Loxoma_cunninghamii                  AGCTCAGGCCGAAACAGGCGAAATCAAGGGACATTACTTAAATGCTACTG
Metaxya_rostrata                     AGCTCAGGCTGAAACGGGCGAAATTAAGGGGCATTACTTAAATGCTACTG
Azolla_caroliniana                   GTCTCTGGCCGAAACGGGCGAAATCAAAGGACATTACTTGAACGCCACTG

                                     701                                              750
Dicksonia_antarctica_Hasebe          CAGGTACTTGTGAAGAAATGATGAAAAGAGCCGTTTTTGCTAGAGAATTG
Dicksonia_antarctica_Wolf            CAGGTACTTGTGAAGAAATGATGAAAAGAGCCGTTTTTGCTAGAGAATTG
Dic_antarctica_Vic                   CAGGTACTTGTGAAGAAATGATGAAAAGAGCCGTTTTTGCTAGAGAATTG
Dic_fibrosa                          CAGGTACTTGTGAAGAAATGATGAAAAGAGCCGTTTTTGCTAGAGAATTG
Dic_sellowiana_SA                    CAGGTACTTGTGAAGAAATGATGAAAAGAGCCGTTTTTGCTAGAGAATTG
Dic_sellowiana_Chile                 CAGGTACTTGTGAAGAAATGATGAAAAGAGCCGTTTTTGCTAGAGAATTG
Dic_arborescens                      CAGGTACTTGTGAAGAAATGATGAAAAGAGCCGTTTTTGCTAGAGAATTG
Dic_baudouinii                       CAGGTACTTGTGAAGAAATGATGAAAAGAGCTGTTTTTGCTAGAGAATTG
Dic_squarrosa                        CAGGTACTTGTGAAGAAATGATGAAAAGAGCTGTTTTTGCTAGAGAATTG
Dic_lanata                           CAGGTACTTGTGAAGAAATGATGAAAAGAGCTGTTTTTGCTAGAGAATTG
Lophosoria_quadripinnata             CAGGTACTTGTGAAGAAATGATGAAAAGAGCCGTTTTTGCTAGAGAATTG
Calochlaena_villosa                  CAGGTACTTGTGAAGAGATGTTGAAAAGAGCCGTTTTTGCTAGAGAATTG
Culcita_dubia                        CAGGTACTTGTGAAGAGATGTTGAAAAGAGCCGTTTTTGCTAGAGAATTG
Culcita_conniifolia                  CAGGTACTTGTGAAGAGATGTTGAAAAGAGCCGTTTTTGCTAGAGAATTG
Cibotium_glaucum                     CAGGTACGTGTGAAGAAATGATGAAAAGAGCAGTTTTTGCTAGAGAATTG
Cibotium_barometz                    CAGGTACGTGTGAAGAAATGATGAAAAGAGCAGTTTTTGCTAGAGAATTG
Sphaeropteris                        CGGGTACGTGTGAAGAAATGTTGAAAAGAGCCCAATTTGCTAGAGAATTG
Sphaeropteris_lepifera               CGGGTACGTGTGAAGAAATGTTGAAAAGAGCCTGTTTTTGCTAGAGAATTG
Sphaeropteris_cooperi                CGGGTACGTGTGAAGAAATGTTGAAAAGAGCCCAATTTGCTAGAGAATTG
Sph_excelsa                          CGGGTACGTGTGAAGAAATGTTGAAAAGAGCCCAATTTGCTAGAGAATTG
Sph_medullaris                       CGGGTACGTGTGAAGAAATGTTGAAAAGAGCCTGTTTTTGCTAGAGAATTG
Als_tricolor                         CAGGTACGTGTGAAGAAATGATGAAAAGGGCCCGTTTTGCTAGAGAATTG
Als_smithii                          CAGGTACGTGTGAAGAAATGTTGAAAAGGGCCCAATTTGCTAGAGAATTG
Hymenophyllopsis_hymenophylloi       CGGGTACGTGCGAAGAAATGTTGAAAAGAGCCCGTTTTGCTAGAGAATTG
Hymenophyllopsis_dejecta             CAGGTACGTGTGAAGAAATGTTGAAAAGAGCCCGTTTTGCTAGAGAATTG
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Plagiogyria_japonica                CAGGTACGTGTGAAGAACCGTCGAAAAGAGCTGTTTTTGCTAGGGAATTG
Loxoma_cunninghamii                 CAGGTACGTGTGAAGAAATGATGAAAAGAGCCGTTTTTGCTAGAGAATTG
Metaxya_rostrata                    CAGGTACATGTGAAGAAATGTTGAAAAGAGCCGTTTTTGCTAGAGAATTG
Azolla_caroliniana                  CAGGTACATGCGAAGAAATGCTAAAAAGAGCTCAATTCGCTAGAGAGTTG

                                    751                                         800
Dicksonia_antarctica_Hasebe         GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Dicksonia_antarctica_Wolf           GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Dic_antarctica_Vic                  GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Dic_fibrosa                         GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Dic_sellowiana_SA                   GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Dic_sellowiana_Chile                GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Dic_arborescens                     GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Dic_baudouinii                      GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Dic_squarrosa                       GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Dic_lanata                          GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Lophosoria_quadripinnata            GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Calochlaena_villosa                 GGGGTACCCATAGTCATGCATGACTATTTGACCGGAGGTTTTACCGCAAA
Culcita_dubia                       GGGGTACCCATAGTCATGCATGACTATTTGACCGGAGGTTTTACCGCAAA
Culcita_conniifolia                 GGGGTACCCATAGTCATGCATGACTATTTGACCGGAGGTTTTACCGCAAA
Cibotium_glaucum                    GGGGCACCAATTGTAATGCATGACTATCTAACCGGAGGGTTTACCGCAAA
Cibotium_barometz                   GGGGTACCAATTATAATGCATGACTATCTAACCGGAGGGTTTACCGCAAA
Sphaeropteris                       GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Sphaeropteris_lepifera              GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Sphaeropteris_cooperi               GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Sph_excelsa                         GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Sph_medullaris                      GGGGTACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Als_tricolor                        GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Als_smithii                         GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Hymenophyllopsis_hymenophylloi      GGGGCACCAATTGTAATGCATGACTATCTAACCGGAGGGTTTACCGCAAA
Hymenophyllopsis_dejecta            GGGGCACCAATTGTAATGCATGACTATCTGACCGGAGGGTTTACCGCAAA
Plagiogyria_japonica                GGAGCACCAATTGTAATGCATGACTACCTGACCGGAGGGTTCACCGCAAA
Loxoma_cunninghamii                 GGAAGCACAATTGTAATGCATGACTACCTGACCGGAGGGTTCACCGCAAA
Metaxya_rostrata                    GGGGGCACAATTGTAATGCATGACTATCTAACCGAGGGTTTTACTGCAAA
Azolla_caroliniana                  GGTGCACCAATCGTCATGCATGACTACCTGACCGGAGGTTTTACTGCAAA

                                    801                                         850
Dicksonia_antarctica_Hasebe         CACTAGCTTGGCTTTTTACTGTCGAGACAATGGGCTGCTTCTTCACATTC
Dicksonia_antarctica_Wolf           CACTAGCTTGGCTTTTTACTGTCGAGACAATGGGCTGCTTCTTCACATTC
Dic_antarctica_Vic                  CACTAGCTTGGCTTTTTACTGTCGAGACAATGGGCTGCTTCTTCACATTC
Dic_fibrosa                         CACTAGCTTGGCTTTTTACTGTCGAGACAATGGGCTGCTTCTTCACATTC
Dic_sellowiana_SA                   CACTAGCTTGGCTTTTTACTGTCGAGACAATGGGCTGCTTCTTCACATTC
Dic_sellowiana_Chile                CACTAGCTTGGCTTTTTACTGTCGAGACAATGGGCTGCTTCTTCACATTC
Dic_arborescens                     CACTAGCTTGGCTTTTTACTGTCGAGACAATGGGCTGCTTCTTCACATTC
Dic_baudouinii                      CACTAGCTTGGCTTTTTACTGTCGAGACAATGGGCTGCTTCTTCACATTC
Dic_squarrosa                       CACTAGCTTGGCTTTTTACTGTCGAGACAATGGGCTGCTTCTTCACATTC
Dic_lanata                          CACTAGCTTGGCTTTTTACTGTCGAGACAATGGGCTGCTTCTTCACATTC
Lophosoria_quadripinnata            TACTAGCTTGGCTTTTTATTGTCGAGACAATGGGCTGCTTCTTCACATTC
Calochlaena_villosa                 TACGAGCTTGGCTTTTTATTGTCGAGACAATGGGCGTCTTCTTCACATTC
Culcita_dubia                       TACGAGCTTGGCTTTTTATTGTCGAGACAATGGGCTGCTTCTTCACATTC
Culcita_conniifolia                 TACGAGCTTGGCTTTTTATTGTCGAGACAATGGGCTGCTTCTTCACATTC
Cibotium_glaucum                    TACTAGCTTGGCCTTTTATTGTCGAGACAATGGGCTGCTTCTTCACATTC
Cibotium_barometz                   TACTAGCTTGGCCTTTTATTGTCGAGACAATGGGCTGCTTCTTCACATTC
Sphaeropteris                       CACTAGCTTGGCCTTCTATTGCCGAGATAATGGGCTGCTTCTTCACATTC
Sphaeropteris_lepifera              CACTAGCTTGGCCTTCTATTGCCGAGATAATGGGCTGCTTCTTCACATTC
Sphaeropteris_cooperi               CACTAGCTTGGCCTTCTATTGCCGAGATAATGGGCTGCTTCTTCACATTC
Sph_excelsa                         CACTAGCTTGGCCTTCTATTGCCGAGATAATGGGCTGCTTCTTCACATTC
Sph_medullaris                      CACTAGCTTGGCCTTCTATTGCCGAGATAATGGGCTGCTTCTTCACATTC
Als_tricolor                        TACTAGCTTGGCCTTCTATTGTCGAGATAATGGGCTGCTTCTTCACATTC
Als_smithii                         TACTAGCTTGGCCTTCTATTGTCGAGATAATGGGCTGCTTCTGCACATTC
Hymenophyllopsis_hymenophylloi      TACTACCTTGGCCTTCTATTGCCGAGATAATGGGCTGCTTCTTCACATTC
Hymenophyllopsis_dejecta            TACTAGCTTGGCCTTCTATTGCCGAGATAATGGGCTGCTTCTTCACATTC
Plagiogyria_japonica                TACTAGCTTGGCCTTTTATTGTCGAGACAATGGGCTGCTTCTTCACATTC
Loxoma_cunninghamii                 TACTAGCTTGGCCTTTTACTGTCGAGACAATGGGCTGCTTCTTCACATTC
Metaxya_rostrata                    TACTAGTTTGGCTTTTTACTGTCGAGACAATGGACTGCTTCTTCACATTC
Azolla_caroliniana                  CACTAGCTTGGCTTTCTACTGCCGAGACAATGGGCTACTTCTTCACATTC

                                    851                                         900
Dicksonia_antarctica_Hasebe         ACCGTGCGATGCATGCTGTCATCGATAGACAGAGAAATCACGGTATACAT
Dicksonia_antarctica_Wolf           ACCGTGCGATGCATGCTGTCATCGATAGACAGAGAAATCACGGTATACAT
Dic_antarctica_Vic                  ACCGTGCGATGCATGCTGTCATCGATAGACAGAGAAATCACGGTATACAT
Dic_fibrosa                         ACCGTGCGATGCATGCTGTCATCGATAGACAGAGAAATCACGGTATACAT
Dic_sellowiana_SA                   ACCGTGCGATGCATGCTGTCATCGATAGACAGAGAAATCACGGTATACAT
Dic_sellowiana_Chile                ACCGTGCGATGCATGCTGTCATCGATAGACAGAGAAATCACGGTATACAT
Dic_arborescens                     ACCGTGCGATGCATGCTGTCATCGATAGACAGAGAAATCACGGTATACAT
Dic_baudouinii                      ACCGTGCGATGCATGCTGTCATCGATAGACAAAGAAATCACGGTATACAT
Dic_squarrosa                       ACCGTGCGATGCATGCTGTCATCGATAGACAAAGAAATCACGGTATACAT
Dic_lanata                          ACCGTGCGATGCATGCTGTCATCGATAGACAAAGAAATCACGGTATACAT
Lophosoria_quadripinnata            ACCGTGCGATGCATGCTGTCATCGATAGACAGAGAAATCACGGTATACAT
Calochlaena_villosa                 ACCGTGCGATGCATGCTGTCATCGATAGACAAAAAAATCACGGTATACAT
Culcita_dubia                       ACCGTGCGATGCATGCTGTCATCGATAGACAAAAAAATCACGGTATACAT
Culcita_conniifolia                 ACCGTGCGATGCATGCTGTCATCGATAGACAAAAAAATCACGGTATACAT
Cibotium_glaucum                    ACCGTGCAATGCATGCTGTCATCGATAGACAGAAAAATCACGGTATGCAT
Cibotium_barometz                   ACCGTGCGATGCATGCTGTCATCGATAGACAGAAAAATCACGGTATGCAT
Sphaeropteris                       ACCGTCGAATGCATGCTGTCATCGATAGACAGAAAAATCACGGTATACAT
Sphaeropteris_lepifera              ACCGTGCAATGCATGCTGTCATCGATAGACAGAAAAATCACGGTATACAT
Sphaeropteris_cooperi               ACCGTGCAATGCATGCTGTCATCGATAGACAGAAAAATCACGGTATACAT
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Sph_excelsa                          ACCGTGCAATGCATGCTGTCATCGATAGACAGAAAAATCACGGTATACAT
Sph_medullaris                       ACCGTGCAATGCATGCTGTCATCGATAGACAGAAAAATCACGGTATACAT
Als_tricolor                         ACCGTGCAATGCATGCTGTCATCGATAGACAGAAAAATCACGGTATACAT
Als_smithii                          ACCGTGCAATGCATGCTGTCATCGATAGACAGAGAAATCACGGTATACAT
Hymenophyllopsis_hymenophylloi       ACCGTGCAATGCATGCTGTCATCGATAGACAGAAAAATCACGGTATGCAT
Hymenophyllopsis_dejecta             ACCGTGCAATGCATGCTGTCATCGATAGACAGAGAAATCACGGTATACAT
Plagiogyria_japonica                 ACCGTGCGATGCATGCCGTCATTGATAGACAAAAAAACCACGGTATGCAT
Loxoma_cunninghamii                  ACCGTGCGATGCATGCCGTCATCGATAGACAGAGAAATCACGGTATACAT
Metaxya_rostrata                     ACCGTGCAATGCATGCTGTCATCGATAGACAGAGAAATCACGGTATACAT
Azolla_caroliniana                   ACCGCGCAATGCATGCTGTCATCGATAGACAGAGAAATCATGGTATACAT

                                     901                                              950
Dicksonia_antarctica_Hasebe          TTTCGTGTATTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Dicksonia_antarctica_Wolf            TTTCGTGTATTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Dic_antarctica_Vic                   TTTCGTGTATTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Dic_fibrosa                          TTTCGTGTATTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Dic_sellowiana_SA                    TTTCGTGTATTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Dic_sellowiana_Chile                 TTTCGTGTATTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Dic_arborescens                      TTTCGTGTATTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Dic_baudouinii                       TTTCGTGTATTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Dic_squarrosa                        TTTCGTGTATTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Dic_lanata                           TTTCGTGTATTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Lophosoria_quadripinnata             TTTCGTGTATTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Calochlaena_villosa                  TTCCGTGTATTAGCCAAAGCTTTACGTATGTCCGGTGGAGATCATATNCA
Culcita_dubia                        TTCCGTGTATTAGCCAAAGCTTTACGTATGTCCGGTGGAGATCATATTCA
Culcita_conniifolia                  TTCCGTGTATTAGCCAAAGCTTTACGTATGTCCGGTGGAGATCATATTCA
Cibotium_glaucum                     TTTCGTGTGTTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Cibotium_barometz                    TTTCGTGTGTTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Sphaeropteris                        TTTCGTGTATTAGCAAAAGCATTACGTATGTCCGGTGGGGATCATGTTCA
Sphaeropteris_lepifera               TTTCGTGTATTAGCAAAAGCATTACGTATGTCCGGTGGGGATCATGTTCA
Sphaeropteris_cooperi                TTTCGTGTATTAGCAAAAGCATTACGTATGTCCGGTGGGGATCATGTTCA
Sph_excelsa                          TTTCGTGTATTAGCAAAAGCATTACGTATGTCCGGTGGGGATCATGTTCA
Sph_medullaris                       TTTCGTGTATTAGCAAAAGCATTACGTATGTCCGGTGGGGATCATGTTCA
Als_tricolor                         TTTCGTGTATTAGCAAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Als_smithii                          TTTCGTGTATTAGCAAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Hymenophyllopsis_hymenophylloi       TTTCGTGTATTAGCAAAAGCATTACGTATGTCCGGTGGAGATCATATCCA
Hymenophyllopsis_dejecta             TTTCGTGTATTAGCAAAAGCATTACGTATGTCCGGTGGAGATCATATTCA
Plagiogyria_japonica                 TTTCGTGTATTAGCCAAAGCATTACGTATGTCCGGTGGAGATCATGTTCA
Loxoma_cunninghamii                  TTTCGTGTATTAGCCAAAGCATTACGTATGCCTCGGTGGAGATCACATTCA
Metaxya_rostrata                     TTTCGTGTATTAGCCAAAGCATTACGCATGTCTGGTGGAGACCATATCCA
Azolla_caroliniana                   TTCCGCGTGTTAGCCAAAGCATTACGTATGTCTGGTGGGGACCATATCCA

                                     951                                             1000
Dicksonia_antarctica_Hasebe          CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Dicksonia_antarctica_Wolf            CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Dic_antarctica_Vic                   CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Dic_fibrosa                          CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Dic_sellowiana_SA                    CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Dic_sellowiana_Chile                 CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Dic_arborescens                      CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Dic_baudouinii                       CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Dic_squarrosa                        CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Dic_lanata                           CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Lophosoria_quadripinnata             CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Calochlaena_villosa                  CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTTACTTTGG
Culcita_dubia                        CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTTACTTTGG
Culcita_conniifolia                  CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTTACTTTGG
Cibotium_glaucum                     CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Cibotium_barometz                    CGTGGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Sphaeropteris                        CTCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGTGAAGTTACCTTGG
Sphaeropteris_lepifera               CTCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Sphaeropteris_cooperi                CTCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGTGAAGTTACCTTGG
Sph_excelsa                          CTCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGTGAAGTTACCTTGG
Sph_medullaris                       CTCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Als_tricolor                         CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Als_smithii                          CGCTGGGACTGTAGTAGGCAAACTAGAGGGAGAACGAGAAGTCACTTTGG
Hymenophyllopsis_hymenophylloi       CGCTGGGACTGTAGTAGGCAAATTAGAGGGAGAACGAGAAGTCACTCTGG
Hymenophyllopsis_dejecta             CGCTGGGACTGTAGTAGGCAAATTAGAGGGAGAACGAGAAGTCACTCTGG
Plagiogyria_japonica                 CGCCGGGACTGTAGTAGGTAAACTGGAGGGAGAACGAGAAGTCACTTTGG
Loxoma_cunninghamii                  CGCTGGGACTGTAGTTGGTAAACTAGAGGGAGAACGAGAAGTTACTTTGG
Metaxya_rostrata                     CGCTGGAACTGTAGTGGGTAAATTAGAGGGAGAACGAGACGTCACTTTGG
Azolla_caroliniana                   CTCCGGAACCGTAGTAGGTAAACTAGAAGGAGAGCGAGAAGTAACTCTGG

                                     1001                                            1050
Dicksonia_antarctica_Hasebe          GCTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Dicksonia_antarctica_Wolf            GCTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Dic_antarctica_Vic                   GCTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Dic_fibrosa                          GCTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Dic_sellowiana_SA                    GCTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Dic_sellowiana_Chile                 GCTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Dic_arborescens                      GCTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Dic_baudouinii                       GCTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGATCGTAGCCGC
Dic_squarrosa                        GCTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGATCGTAGCCGC
Dic_lanata                           GCTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGATCGTAGCCGC
Lophosoria_quadripinnata             GCTTTGTCGATTTGCTTCGCGACGATTATATCGAAAAAGACCGTAGCCGC
Calochlaena_villosa                  GCTTTGTCGATTTGCTTCGCGATGATTACATCGAAAAAGACCGTAGCCGC
Culcita_dubia                        GCTTTGTCGATTTGCTTCGCGATGATTACATCGAAAAAGACCGTAGCCGC
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Culcita_conniifolia                    GCTTTGTCGATTTGCTTCGCGATGATTACATCGAAAAAGACCGTAGCCGC
Cibotium_glaucum                       GMTTTGTCGATTTGCTTCGMGACGATGATATCGAAAAAGACCGTAGCCGC
Cibotium_barometz                      GCTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Sphaeropteris                          GTTTTGTCGATTTNCTTCGCGNCGATTATATTGAAAAAGACCGTAGCCGC
Sphaeropteris_lepifera                 GTTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Sphaeropteris_cooperi                  GTTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Sph_excelsa                            GTTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Sph_medullaris                         GTTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Als_tricolor                           GTTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Als_smithii                            GTTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Hymenophyllopsis_hymenophylloi         GTTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Hymenophyllopsis_dejecta               GTTTTGTCGATTTGCTTCGCGACGATTATATTGAAAAAGACCGTAGCCGC
Plagiogyria_japonica                   GCTTTGTCGATTTTCTTCGCGACGATTATATAGAAAAAGACCGTAGCCGC
Loxoma_cunninghamii                    GCTTTGTCGATTTGCTTCGAGACGATTATATCGAGAAAGACCGTAGCCGC
Metaxya_rostrata                       GATTTGTTGATTTGCTCCGCGAGGATTATGTCGAAAAAGATCGTAGCCGT
Azolla_caroliniana                     GTTTTGTCGATCTACTTCGCGACGATTACATTGAAAAGGACCGTAGCCGC

                                       1051                                          1100
Dicksonia_antarctica_Hasebe            GGCATCTATTTCACCCAAGATTGGGTATCGATGCCAGGCGTACTTCCCGT
Dicksonia_antarctica_Wolf              GGCATCTATTTCACCCAAGATTGGGTATCGATGCCAGGCGTACTTCCCGT
Dic_antarctica_Vic                     GGCATCTATTTCACCCAAGATTGGGTATCGATGCCAGGCGTACTTCCCGT
Dic_fibrosa                            GGCATCTATTTCACCCAAGATTGGGTATCGATGCCAGGCGTACTTCCCGT
Dic_sellowiana_SA                      GGCATCTATTTCACCCAAGATTGGGTATCGATGCCAGGCGTACTTCCCGT
Dic_sellowiana_Chile                   GGCATCTATTTCACCCAAGATTGGGTATCGATGCCAGGCGTACTTCCCGT
Dic_arborescens                        GGCATCTATTTCACCCAAGATTGGGTATCTATGCCAGGCGTACTTCCCGT
Dic_baudouinii                         GGCATCTATTTCACCCAAGATTGGGTATCTATGCCAGGCGTACTTCCCGT
Dic_squarrosa                          GGCATCTATTTCACCCAAGATTGGGTATCTATGCCAGGCGTACTTCCCGT
Dic_lanata                             GGCATCTATTTCACCCAAGATTGGGTATCTATGCCAGGCGTACTTCCCGT
Lophosoria_quadripinnata               GGCATCTATTTTACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCCGT
Calochlaena_villosa                    GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCTGT
Culcita_dubia                          GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCTGT
Culcita_conniifolia                    GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCTGT
Cibotium_glaucum                       GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCCGT
Cibotium_barometz                      GGCGTCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCCGT
Sphaeropteris                          GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCCGT
Sphaeropteris_lepifera                 GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCCGT
Sphaeropteris_cooperi                  GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCCGT
Sph_excelsa                            GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCCGT
Sph_medullaris                         GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCCGT
Als_tricolor                           GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTATTTCCCGT
Als_smithii                            GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCCGT
Hymenophyllopsis_hymenophylloi         GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCCGT
Hymenophyllopsis_dejecta               GGCATCTATTTCACCCAAGATTGGGTATCTATGCCGGGCGTACTTCCCGT
Plagiogyria_japonica                   GGTGTCTATTTCACCCAAGACTGGGTCTCTATGCCGGGGTGTACTTCCCGT
Loxoma_cunninghamii                    GGTATTTATTTCACCCGAGATTGGGTATCCATGCCGGGCGTACTTCCCGT
Metaxya_rostrata                       GGCATCTATTTCACTCAAGATTGGGTATCTATGCCGGGCGTAATTCCCGT
Azolla_caroliniana                     GGTATCTATTTCACCCAAGATTGGGTATCTATGCCAGGAGTATTGCCTGT

                                       1101                                          1150
Dicksonia_antarctica_Hasebe            AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATCT
Dicksonia_antarctica_Wolf              AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATCT
Dic_antarctica_Vic                     AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATCT
Dic_fibrosa                            AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATCT
Dic_sellowiana_SA                      AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATCT
Dic_sellowiana_Chile                   AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATCT
Dic_arborescens                        AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATCT
Dic_baudouinii                         AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATCT
Dic_squarrosa                          AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATCT
Dic_lanata                             AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATCT
Lophosoria_quadripinnata               AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATCT
Calochlaena_villosa                    AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATAT
Culcita_dubia                          AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATAT
Culcita_conniifolia                    AGCTTCGGGGGGTATCCATGTCTGGCATATGCCTGCTCTAACCGAAATAT
Cibotium_glaucum                       AGCTTCGGGGGGTATCCACGTCTGGCATATACCTGCTCTAACCGAAATCT
Cibotium_barometz                      AGCTTCGGGGGGTATCCACGTCTGGCATATGCCTGCTCTAACCGAAATCT
Sphaeropteris                          AGCTTCGGGGGGTATCCACGTATGGCATATGCCTGCTCTAACCGAAATCT
Sphaeropteris_lepifera                 AGCTTCGGGGGGTATCCACGTATGGCATATGCCTGCTCTAACCGAAATCT
Sphaeropteris_cooperi                  AGCTTCGGGGGGTATCCACGTATGGCATATGCCTGCTCTACCCGAAATCT
Sph_excelsa                            AGCTTCGGGGGGTATCCACGTATGGCATATGCCTGCTCTAACCGAAATCT
Sph_medullaris                         AGCTTCGGGGGGTATCCACGTATGGCATATGCCTGCTCTAACCGAAATCT
Als_tricolor                           AGCTTCGGGGGGTATCCACGTATGGCATATGCCTGCTCTAACCGAAATCT
Als_smithii                            AGCTTCGGGGGGTATCCACGTATGGCATATGCCTGCTCTAACCGAAATCT
Hymenophyllopsis_hymenophylloi         AGCTTCGGGGGGTATCCACGTATGGCATATGCCTGCTCTAACTGAAATCT
Hymenophyllopsis_dejecta               AGCTTCGGGGGGTATCCACGTATGGCATATGCCTGCTCTAACCGAAATCT
Plagiogyria_japonica                   AGCTTCGGGGGGTATCCACGTCTGGCATATGCCCGCTCTAACCGAAATCT
Loxoma_cunninghamii                    AGCTTCGGGGGGTATCCACGTCTGGCATATGCCTGCTCTAACCGAAATCT
Metaxya_rostrata                       AGCTTCGGGGGGTATTCACGTTTGGCATATGCCCGCTCTAACCGAGATTT
Azolla_caroliniana                     AGCTTCAGGTGGTATCCACGTATGGCACATGCCCGCTCTAACCGAGATTT

                                       1151                                          1200
Dicksonia_antarctica_Hasebe            TCGGAGACGATTCCGTGTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Dicksonia_antarctica_Wolf              TCGGAGACGATTCCGTGTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Dic_antarctica_Vic                     TCGGAGACGATTCCGTGTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Dic_fibrosa                            TCGGAGACGATTCCGTGTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Dic_sellowiana_SA                      TCGGAGACGATTCCGTGTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Dic_sellowiana_Chile                   TCGGAGACGATTCCGTGTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Dic_arborescens                        TCGGAGACGATTCCGTGTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
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Dic_baudouinii                      TCGGAGACGATTCCGTGTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Dic_squarrosa                       TCGGAGACGATTCCGTGTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Dic_lanata                          TCGGAGACGATTCCGTGTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Lophosoria_quadripinnata            TCGGAGACGATTCCGTCTTACAGTTCGGTGGAGGAACCTTGGGACATCCT
Calochlaena_villosa                 TCGGAGACGATTCCGTCTTACAGTTCGGCGGCGGAACTTTGGGACATCCT
Culcita_dubia                       TCGGAGACGATTCCGTCTTACAGTTCGGCGGCGGAACTTTGGGACATCCT
Culcita_conniifolia                 TCGGAGACGATTCCGTCTTACAGTTCGGCGGCGGAACTTTGGGACATCCT
Cibotium_glaucum                    TCGGAGACGACTCTGTCTTACAGTTCGGTGGAGGAACCCTGGGACATCCT
Cibotium_barometz                   TCGGAGACGATTCTGTCTTACAGTTCGGTGGAGGAACCGTAGGACATCCT
Sphaeropteris                       TCGGAGACGATTCTGTCTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Sphaeropteris_lepifera              TCGGAGACGATTCTGTCTTACAGTTCGGCGGAGGAACCCTGGGACATCCT
Sphaeropteris_cooperi               TCGGAGACGATTCTGTCTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Sph_excelsa                         TCGGAGACGATTCTGTCTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Sph_medullaris                      TCGGAGACGATTCTGTCTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Als_tricolor                        TCGGAGACGATTCTGTCTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Als_smithii                         TCGGGGACGATTCTGTCTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Hymenophyllopsis_hymenophylloi      TCGGAGACGATTCTGTCTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Hymenophyllopsis_dejecta            TCGGAGACGATTCTGTCTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Plagiogyria_japonica                TCGGAGACGATTCCGTCTTACAGTTTGGCGGGGGAACCCTCGGACATCCC
Loxoma_cunninghamii                 TCGGAGATGATTCTGTCTTACAGTTCGGCGGAGGAACCTTGGGACATCCT
Metaxya_rostrata                    TCGGGGACGATTCCGTCTTACAGTTCGGGGGAGGAACCTTGGGACATCCT
Azolla_caroliniana                  TTGGGGACGATTCCGTATTACAATTTGGTGGAGGAACCCTAGGCCATCCT

                                    1201                                          1250
Dicksonia_antarctica_Hasebe         TGGGGAAATGCGCCCGGTGCTGTAGCTAATCGAGTCGCGTCAGAGGCGTG
Dicksonia_antarctica_Wolf           TGGGGAAATGCGCCCGGTGCTGTAGCTAATCGAGTCGCGTCAGAGGCTTG
Dic_antarctica_Vic                  TGGGGAAATGCGCCCGGTGCTGTAGCTAATCGAGTCGCGTCAGAGGCTTG
Dic_fibrosa                         TGGGGAAATGCGCCCGGTGCTGTAGCTAATCGAGTCGCGTCAGAGGCTTG
Dic_sellowiana_SA                   TGGGGAAATGCGCCCGGTGCTGTAGCTAATCGAGTCGCGTCAGAGGCTTG
Dic_sellowiana_Chile                TGGGGAAATGCGCCCGGTGCTGTAGCTAATCGAGTCGCGTCAGAGGCTTG
Dic_arborescens                     TGGGGAAATGCGCCCGGTGCTGTAGCTAATCGAGTTGCGTTAGAGGCTTG
Dic_baudouinii                      TGGGGAAATGCGCCCGGTGCTGTAGCCAATCGAGTCGCGTCAGAGGCTTG
Dic_squarrosa                       TGGGGAAATGCGCCCGGTGCTGTAGCTAATCGAGTCGCGTCAGAGGCTTG
Dic_lanata                          TGGGGAAATGCGCCCGGTGCTGTAGCTAATCGAGTCGCGTCAGAGGCTTG
Lophosoria_quadripinnata            TGGGGAAATGCACCCGGTGCCGTAGCTAATCGAGTCGCGTTAGAGGCTTG
Calochlaena_villosa                 TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTCAGAGGCTTG
Culcita_dubia                       TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTCAGAGGCTTG
Culcita_conniifolia                 TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTCAGAGGCTTG
Cibotium_glaucum                    TNGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTTAGAGGCTTG
Cibotium_barometz                   TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTTAGAGGCTTG
Sphaeropteris                       TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTTAGAGGCTTG
Sphaeropteris_lepifera              TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTTAGAGGCTTG
Sphaeropteris_cooperi               TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTTAGAGGCTTG
Sph_excelsa                         TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTTAGAGGCTTG
Sph_medullaris                      TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTTAGAGGCTTG
Als_tricolor                        TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTTAGAGGCTTG
Als_smithii                         TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTCAGAGGCTTG
Hymenophyllopsis_hymenophylloi      TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTTAGAGGCTTG
Hymenophyllopsis_dejecta            TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTTAGAGGCTTG
Plagiogyria_japonica                TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTCGCGTTAGAGGCTTG
Loxoma_cunninghamii                 TGGGGAAATGCGCCCGGTGCCGTAGCTAATCGAGTTGCGTTAGAAGCT--
Metaxya_rostrata                    TGGGGAAATGCGCCCGGTGCCGTAGCCAATCGAGTCGCATCAGAGGCTTG
Azolla_caroliniana                  TGGGGGAACGCACCCGGTGCTGTAGCTAACAGAGTGGCTTTGGAGGCTTG

                                    1251                                          1300
Dicksonia_antarctica_Hasebe         TGTACAAGCTCGTAATGAGGGCCGTGACCGTGCTCGTGAAGGTAATGAAA
Dicksonia_antarctica_Wolf           TGTACAAGCTCGTAATGAGGGCCGTGACCTTGCTCGTGAAGGTAATGAAA
Dic_antarctica_Vic                  TGTACAAGCTCGTAATGAGGGCCGTGACCTTGCTCGTGAAGGTAATGAAA
Dic_fibrosa                         TGTACAAGCTCGTAATGAGGGCCGTGACCTTGCTCGTGAAGGTAATGAAA
Dic_sellowiana_SA                   TGTACAAGCTCGTAATGAGGGCCGTGACCTTGCTCGTGAAGGTAATGAAA
Dic_sellowiana_Chile                TGTACAAGCTCGTAATGAGGGCCGTGACCTTGCTCGTGAAGGTAATGAAA
Dic_arborescens                     TGTACAAGCTCGTAATGAGGGCCGTGACCTTGCTCGTGAAGGTAATGAAA
Dic_baudouinii                      TGTACAAGCCCGTAATGAGGGCCGTGACCTTGCTCGTGAAGGTAATGAAA
Dic_squarrosa                       TGTACAAG----------------------------------------
Dic_lanata                          TGTACAAGCTCGTAATGAGG----------------------------
Lophosoria_quadripinnata            TGTACAAGCTCGTAATGAGGGCCGTGACCTTGCTCGTGAAGGTAATGAGA
Calochlaena_villosa                 TGTACAAGCTCGTAATGAGGGCCGTGACCTTGCCCGTGAAGGTAATGAAA
Culcita_dubia                       TGTACAAGCTCGTAATGAGGGCCGTGACCTTGCCCGTGAAGGTAATGACA
Culcita_conniifolia                 TGTACAAGCTCGTAATGAGGGCCGTGACCTTGCCCGTGAAGGTAATGAAA
Cibotium_glaucum                    TGTACAAGCTCGTAATGAGGGCCGNGACCTCGCTCGTGAAGGTAATNATA
Cibotium_barometz                   TGTACAAGCTCGTAATGAGGGCCGTGACCTCGC----------------
Sphaeropteris                       TGTACAAGCTCGTAATGAAGGCCGTGACCTTGCTCGTGAAGGTAATGAGA
Sphaeropteris_lepifera              TGTACAAGCTCGTAATGAAGCTCGCGATCTTGCTCGTG------------
Sphaeropteris_cooperi               TGTACAAGCTCGTAATGAAGGCCGTGACCTTGCTCGTGAAGGTAATGAGA
Sph_excelsa                         TGTACAAGCTCGTAATGAAGGCCGTGACCTTGCTCGTGAAGGTAATGAGA
Sph_medullaris                      TGTACAAGCTCGTAATGAAGGCCGTGACCTTGCTCGTGAAGGTAATGAGA
Als_tricolor                        TGTACAAGCTCGTAATGAGGGCCGTGACCTTGCTCGTGAAGGTAATGAGA
Als_smithii                         TGTACAAGCTCGTAATGAGGGCCGTGACCTTGCTCGTGAAGGTAATGAGA
Hymenophyllopsis_hymenophylloi      TGTACAAGCTCGTAATGAGGGTCGTGACCTTGCTCGTGAAGGTAATGAGA
Hymenophyllopsis_dejecta            TGTACAAGCTCGTAATGAGGGTCGTGACCTTGCTCGTGAAGGTAATGAGA
Plagiogyria_japonica                TGTACAAGCTCGTAATGAGGGCCGTGACCT--------------------
Loxoma_cunninghamii                 ----------------------------------------------
Metaxya_rostrata                    TGTACAAGCCCGTAATGAGGGCCGCGAC----------------------
Azolla_caroliniana                  TGTTCAAGCCCGTAACGAAGGCCGTGATCTTGCTCGTGAAGGTAACGAGA

                                    1301                                  1334
Dicksonia_antarctica_Hasebe         ----------------------------------
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Dicksonia_antarctica_Wolf          TTATTCGTGAAGCTTGTAAA----------
Dic_antarctica_Vic                 TTATTCGTGAAGCTTGTAAA----------
Dic_fibrosa                        TTATTCGTGAAGCTTGTAAA----------
Dic_sellowiana_SA                  TTATTCGTGAAGCTTGTAAA----------
Dic_sellowiana_Chile               TTATTCGTGAAGCTTGTAAA----------
Dic_arborescens                    TTATTCGTGAAGCTTGTAAA----------
Dic_baudouinii                     TTATTCGTGAAGCTTGTAAA----------
Dic_squarrosa                      ----------
Dic_lanata                         ----------
Lophosoria_quadripinnata           TTATTCGTGAAGCTTGTAAGTG--------
Calochlaena_villosa                TTATTCGCGAAGCTTGTAAGTGGAGT--------
Culcita_dubia                      ----------
Culcita_conniifolia                TTATTCGCGAAGCTTGTAAG----------
Cibotium_glaucum                   TTATTCGTGAAGCTAGTAAG----------
Cibotium_barometz                  ----------
Sphaeropteris                      TTATTCGTGAAGCTAGTCAG----------
Sphaeropteris_lepifera             ----------
Sphaeropteris_cooperi              TTATTCGTGAAGCTAGTCAG----------
Sph_excelsa                        TTATTCGTGAAGCTAGTCAG----------
Sph_medullaris                     TTATTCGTGAAGCTAGTCAG----------
Als_tricolor                       TTATCCGTGAAGCTAGTAAG----------
Als_smithii                        TTATCCGTGAAGCTAGTAAG----------
Hymenophyllopsis_hymenophylloi     TTATCCGTGAAGCTAGTAAGT---------
Hymenophyllopsis_dejecta           TTATCCGTGAAGCTAGTAAGT---------
Plagiogyria_japonica               ----------
Loxoma_cunninghamii                ----------
Metaxya_rostrata                   ----------
Azolla_caroliniana                 TTATTCGTGAGGCTAGTAAGTGGAGTCCCGAACT
```

APPENDIX 4

4.1 DNA sequence alignments

4.1.1 *Dicksonia trnL-trnF* spacer

Sequence Label	Species	Source/GenBank Accession
LanNOma	*Dicksonia lanata*	New Zealand/L.R. Perrie
LanSPir	*Dicksonia lanata*	New Zealand/L.R. Perrie
LanSUre2	*Dicksonia lanata*	New Zealand/L.R. Perrie
LanNTro	*Dicksonia lanata*	New Zealand/L.R. Perrie
Squ	*Dicksonia squarrosa*	New Zealand/R.L. Lewis
Bau	*Dicksonia baudouinii*	New Caledonia/E. Cameron
Arb	*Dicksonia arborescens*	St. Helena/Q. Cronk
Ant2Vic	*Dicksonia antarctica*	Australia/RBG Sydney
SelSA	*Dicksonia sellowiana*	Chile/RBG Sydney
You	*Dicksonia antarctica*	Australia/Fern Society, Nelson
Fib	*Dicksonia fibrosa*	New Zealand/M.F. Large
Dic_berteriana	*Dicksonia sellowiana*	Chile/C. Lehenbeck
LophQuad	*Lophosoria quadripinnata*	Mexico/B.S. Parris

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                1                                                          60
LanNOma           CAACAGAACAGGGTAGAATCCCCGATCAATCTATATATATAGATTTTT-----CACATCC
LanSPir           CAACAGAACAGGGTAGAATCCCCGATCAATCTATATATATAGATTTTT-----CACATCC
LanSUre2          CAACAGAACAGGGTAGAATCCCCGATCAATCTATATATATAGATTTTT-----CACATCC
LanNTro           CAACAGAACAGGGTAGAATCCCCGATCAATCTATATATATAGATTTTT-----CACATCC
Squ               CAACAGAACAGGGTAGAATCCCCGATCAATCTATATATATAGATTTTT-----CACATCC
Bau               CAACAGAACAGGGTAGAATCCCCGATCAATCTATATATATATATTTTT-----CACATCC
Arb               CAACAGAACAGGGTAGAATCCCCGATCAATCTATATATATAGATTTTTTTTTTCAGATCC
Ant2Vic           CAACAGAACAGGGTAGAATCTCCGATCAATCTATATATATAGATTTTT-----CAGATCC
SelSA             CAACAGAACAGGGTAGAATCTCCGATCAATCTATATATATAGATTTTT-----CAGATCC
You               CAACAGAACAGGGTAGAATCTCCGATCAATCTATATATATAGATTTTT-----CAGATCC
Fib               CAACAGAACAGGGTAGAATCTCCGATCAATCTATATATATAGATTTTT-----CAGATCC
Dic_berteriana    CAACAGAACAGGGTAGAATCTCCGATCAATCTATATATATAGATTTTT-----CAGATCC
LophQuad          CAACAGAACAGGGTAGAATCCCCGAACAATCTCTATATAGAGATTGTT-----CGGATCC

                61                                                         120
LanNOma           ATATGGTTCTCTCACTCCTTTGAACTAT-----AGGTAAGGGGTGGCAGGTTTTCTATCT
LanSPir           ATATGGTTCTCTCACTCCTTTGAACTAT-----AGGTAAGGGGTGGCAGGTTTTCTATCT
LanSUre2          ATATGGTTCTCTCACTCCTTTGAACTAT-----AGGTAAGGGGTGGCAGGTTTTCTATCT
LanNTro           ATATGGTTCTCTCACTCCTTTGAACTAT-----AGGTAAGGGGTGGCAGGTTTTCTATCT
Squ               ATATGGTTCTCTCACTCCTTTGAACTAT-----AGGTAAGGGGTGGCAGGTTTTCTATCT
Bau               ATATGGTTCTCTCACTCCTTCGAACTATAGGTTGGGTAAGGGGTGGCAGGTTTTCTATCT
Arb               ATATGGTTCTCTCACTCCTTTGAACTAT-----GGGTAAGGGGTGGCAGGTTTTATATCT
Ant2Vic           ATATGGTTCTCTCACTCCCTTGAACTAT-----GGGTAAGGGGTGGCAGGTTTTATATCT
SelSA             ATATGGTTCTCTCACTCCCTTGAACTAT-----GGGTAAGGGGTGGCAGGTTTTATATCT
You               ATATGGTTCTCTCACTCCCTTGAACTAT-----GGGTAAGGGGTGGCAGGTTTTATATCT
Fib               ATATGGTTCTCTCACTCCCTTGAACTAT-----GGGTAAGGGGTGGCAGGTTTTATATCT
Dic_berteriana    ATATGGTTCTCTCACTCCCTTGAACTAT-----GGGTAAGGGGTGGCAGGTTTTATATCT
LophQuad          ATATGGTTCTCTCACTCCTTTGAACTAT-----AGG-AAGGGATGGTAAGTTTTATATCT

                121                                                        180
LanNOma           TTGACTCGGAGCTCGATCAATTTAGATTACTACCCTGTCCCTTTGAAAGCAGTTTAACAT
LanSPir           TTGACTCGGAGCTCGATCAATTTAGATTACTACCCTGTCCCTTTGAAAGCAGTTTAACAT
LanSUre2          TTGACTCGGAGCTCGATCAATTTAGATTACTACCCTGTCCCTTTGAAAGCAGTTTAACAT
LanNTro           TTGACTCGGAGCTCGATCAATTTAGATTACTACCCTGTCCCTTTGAAAGCAGTTTAACAT
Squ               TTGACTCGGAGCTCGATCAATTTAGATTACTACCCTGTCCCTTTGAAAGCAGTTTAACAT
Bau               TTGACTCGGAGCTCGATCAAATTAGATTACTACCCTGTCCCTTTGAAAGCAGTTTAACAT
Arb               TTGACTCGGAGCTCGATCAATTTAGATTACTACCCTGTCCCTTTGAAAGTAGTTTAACAC
Ant2Vic           TTGACTCGGAGCTCGATCAATTTAGATTACTACCCTGTCCCTTTGAAAGCAGTTTAACAT
SelSA             TTGACTCGGAGCTCGATCAATTTAGATTACTACCCTGTCCCTTTGAAAGCAGTTTAACAT
You               TTGACTCGGAGCTCGATCAATTTAGATTACTACCCTGTCCCTTTGAAAGCAGTTTAACAT
Fib               TTGACTCGGAGCTCGATCAATTTAGATTACTACCCTGTCCCTTTGAAAGCAGTTTAACAT
```

```
Dic_berteriana      TTGACTCGGAGCTCGATCAATTTAGATTACTACCCTGTCCCTTTGAAAGCAGTTTAACAT
LophQuad            TTGACTCAGAGCTCGATCAATTTAGATTAATACCCTGTCCCTTTGAAAGCAGTTTAACAT

                   181                                                      240
LanNOma             TTTATTTGAATATGGATCACAAATGAACCACTCAGCAAACCTAAAGG------GCTGGTT
LanSPir             TTTATTTGAATATGGATCACAAATGAACCACTCAGCAAACCTAAAGG------GCTGGTT
LanSUre2            TTTATTTGAATATGGATCACAAATGAACCACTCAGCAAACCTAAAGG------GCTGGTT
LanNTro             TTTATTTGAATATGGATCACAAATGAACCACTCAGCAAACCTAAAGG------GCTGGTT
Squ                 TTTATTTGAATATGGATCACAAATGAACCACTCAGCAAACCTAAAGG------GCTGGTT
Bau                 TTTATTTGAATATGGATCACAAATGAACCACTCAGCAAACCTAAAGG------GCTGGTT
Arb                 TTTATTTGAGTATGGATCACAAATGAACCACTCAGCAAACCTAAAGG------GCTGGTT
Ant2Vic             TTTATTTGAGTATGGATCACAAATGAACCACTCAGCAAACCTAAAGGTAAAGGGCTGGTT
SelSA               TTTATTTGAGTATGGATCACAAATGAACCACTCAGCAAACCTAAAGGTAAAGGGCTGGTT
You                 TTTATTTGAGTATGGATCACAAATGAACCACTCAGCAAACCTAAAGGTAAAGGGCTGGTT
Fib                 TTTATTTGAGTATGGATCACAAATGAACCACTCAGCAAACCTAAAGGTAAAGGGCTGGTT
Dic_berteriana      TTTATTTGAGTATGGATCACAAATGAACCACTCAGCAAACCTAAAGGTAAAGGGCTGGTT
LophQuad            TTTATTTGAGTATGGATCACAAATGAACCACTCAGCAAGCCTAAAGG------GATGTTT

                   241                                                      300
LanNOma             GAGTGGTTCATTGGAAAACTATAGGAAATATTGAACAAAA----------GGCTATT--T
LanSPir             GAGTGGTTCATTGGAAAACTATAGGAAATATTGAACAAAA----------GGCTATT--T
LanSUre2            GAGTGGTTCATTGGAAAACTATAGGAAATATTGAACAAAA----------GGCTATT--T
LanNTro             GAGTGGTTCATTGGAAAACTATAGGAAATATTGAACAAAA----------GGCTATT--T
Squ                 GAGTGGTTCATTGGAAAACTATAGGAAATATTGAACAAAATTGAACAAAAGGCTATT--T
Bau                 GAGTGGTTCATTGGAAAACTATAGGAAATATTGAACAAAA----------GGCTATT--T
Arb                 GAGTGGTTCATTGGAAAACTATAGGAAATATTGAACAAAA----------GGCTATT--T
Ant2Vic             GAGTGGTTCATTGGAAAACTATAGGAAATATTGAACAAAA----------GGCTATT--T
SelSA               GAGTGGTTCATTGGAAAACTATAGGAAATATTGAACAAAA----------GGCTATT--T
You                 GAGTGGTTCATTGGAAAACTATAGGAAATATTGAACAAAA----------GGCTATT--T
Fib                 GAGTGGTTCATTGAAAAACTATAGGAAATATTGAACAAAA----------GGCTATT--T
Dic_berteriana      GAGTGGTTCATTGGAAAACTATAGGAAATATTGAACAAAA----------GGCTATT--T
LophQuad            GAGCGGTTCATTTGAAAACTATAAGAAATATTGAACAAAA----------GACTATTACT

                   301                                                      360
LanNOma             ATTGTTTTACCGAGGTTATTGGTTCGATTGGGC-------------------------TC
LanSPir             ATTGTTTTACCGAGGTTATTGGTTCGATTGGGC-------------------------TC
LanSUre2            ATTGTTTTACCGAGGTTATTGGTTCGATTGGGC-------------------------TC
LanNTro             ATTGTTTTACCGAGGTTATTGGTTCGATTGGGC-------------------------TC
Squ                 ATTGTTTTACCGAGGTTATTGGTTCGATTGGGC-------------------------TC
Bau                 ATTGTTTTACCGAGGTTATTGGTTCGATTGGGC-------------------------TC
Arb                 ATTGTTTTATCGAGGTTATTGGTTCGATTGGGC-------------------------TC
Ant2Vic             ATTGTTTTACCGAGGTTATTGGTTCGATTGGGC-------------------------TC
SelSA               ATTGTTTTACCGAGGTTATTGGTTCGATTGGGC-------------------------TC
You                 ATTGTTTTACCGAGGTTATTGGTTCGATTGGGC-------------------------TC
Fib                 ATTGTTTTACCGAGGTTATTGGTTCGATTGGGC-------------------------TC
Dic_berteriana      ATTGTTTTACCGAGGTTATTGGTTCGATTGGGC-------------------------TC
LophQuad            ATTGTTTTACCGAGGTTATTGGTTCGATTGGGCAGAGATAGATCGAATAAAAAAGATATC

                   361                                                      420
LanNOma             TGTCTCATTTGGATTTGAGGCATCTCTCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
LanSPir             TGTCTCATTTGGATTTGAGGCATCTCTCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
LanSUre2            TGTCTCATTTGGATTTGAGGCATCTCTCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
LanNTro             TGTCTCATTTGGATTTGAGGCATCTCTCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
Squ                 TGTCTCATTTGGATTTGAGGCATCTCTCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
Bau                 TGTCTCATTTGGATTTGAGGCATCTCTCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
Arb                 TGTCTCATTTGGATTTGAGGCATCTCTCTCCGTAGAAACGTTGGCCGGGATAGCTCAGCC
Ant2Vic             TGTCTCATTTGGATTTGAGGCATCTCTCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
SelSA               TGTCTCATTTGGATTTGAGGCATCTCTCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
You                 TGTCTCATTTGGATTTGAGGCATCTCTCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
Fib                 TGTCTCATTTGGATTTGAGGCATCTCTCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
Dic_berteriana      TGTCTCATTTGGATTTGAGGCATCTCTCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
LophQuad            CGTCTCATTTGGATTTGAGGCAT----CTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC

                   421                443
LanNOma             GGTAGAGCAGAGGACTGAAAATC
LanSPir             GGTAGAGCAGAGGACTGAAAATC
LanSUre2            GGTAGAGCAGAGGACTGAAAATC
LanNTro             GGTAGAGCAGAGGACTGAAAATC
Squ                 GGTAGAGCAGAGGACTGAAAATC
Bau                 GGTAGAGCAGAGGACTGAAAATC
Arb                 GGTAGAGCAGAGGACTGAAAATC
Ant2Vic             GGTAGAGCAGAGGACTGAAAATC
SelSA               GGTAGAGCAGAGGACTGAAAATC
You                 GGTAGAGCAGAGGACTGAAAATC
Fib                 GGTAGAGCAGAGGACTGAAAATC
Dic_berteriana      GGTAGAGCAGAGGACTGAAAATC
LophQuad            GGTAGAGCAGAGGACTGAAAATC
```

4.1.2 *Dicksonia* and Cyatheaceae *18S*

Sequence Label	Species	Source/GenBank Accession
DicArborescens	*Dicksonia arborescens*	St. Helena/Q. Cronk
DicFibrosa	*Dicksonia fibrosa*	New Zealand/M.F. Large
DicLanata	*Dicksonia lanata*	New Zealand/L.R. Perrie
DicAntHybseq	*Dicksonia antarctica*	U18624 (Wolf *et al.* 1994) & Australia/P.J. Lockhart*
LopQuadripinnata	*Lophosoria quadripinnata*	Mexico/B.S. Parris
AlsSmithii	*Alsophila smithii*	New Zealand/M.F. Large
AlsDealbata	*Alsophila tricolor*	New Zealand/R.L. Lewis, M.F. Large
SphMedullaris	*Sphaeropteris medullaris*	New Zealand/R.L. Lewis
Adiantum	*Adiantum raddianum*	U18621 (Wolf *et al.* 1994)

* indicates a GenBank sequence adjusted from partial sequencing of another sample.

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                    1                                                    60
DicArborescens       ----------------------------------------GATTAAGCCATGCATGTGT
DicFibrosa           ----------------------------------------AGATTAAGCCATGCATGTGT
DicLanata            ------------------------------------------------TGCATGTGT
DicAntHybseq         -------------------------------------------------ATGCATGTGT
LopQuadripinnata     ----------------------------------------GATTAAGCCATGCATGTGT
AlsSmithii           ------------------------------------------------CATGTGT
AlsDealbata          ----------------------------GCTTGTCTCAAAGATTAAGCCATGCATGTGT
SphMedullaris        ---------------------------------TCAAAGATTAAGCCATGCATGTGT
Adiantum             AACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTGT

                   61                                                   120
DicArborescens       AAGTATAAACTCTCTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTC
DicFibrosa           AAGTATAAACTCTCTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTC
DicLanata            AAGTATAAACTCTCTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTC
DicAntHybseq         AAGTATAAACTCTCTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTC
LopQuadripinnata     AAGTATAAACTCTCTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTC
AlsSmithii           AAGTATAAACTCTCTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTC
AlsDealbata          AAGTATAAACTCTCTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTC
SphMedullaris        AAGTATAAACTCTCTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTC
Adiantum             AAGTATAAACTCTTTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTC

                   121                                                  180
DicArborescens       TTTGATGGTACCTTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCACCAA
DicFibrosa           TTTGATGGTACCTTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCACCAA
DicLanata            TTTGATGGTACCTTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCACCAA
DicAntHybseq         TTTGATGGTACCTTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCACCAA
LopQuadripinnata     TTTGATGGTACCTTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCACCAA
AlsSmithii           TTTGATGGTACCTTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCACCAA
AlsDealbata          TTTGATGGTACCTTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCACCAA
SphMedullaris        TTTGATGGTACCTTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCACCAA
Adiantum             TTTGATGGTACCTTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCACCAA

                   181                                                  240
DicArborescens       CTCCCGACTTCTGGAAGGGACGCATTTATTAGATAAAAGGCCGATGCGGGCTTGCCCGGT
DicFibrosa           CTCCCGACTTCTGGAAGGGACGCATTTATTAGATAAAAGGCCGATGCGGGCTTGCCCGGT
DicLanata            CTCCCGACTTCTGGAAGGGACGCATTTATTAGATAAAAGGCCGATGCGGGCTTGCCCGGT
DicAntHybseq         CTCCCGACTTCTGGAAGGGACGCATTTATTAGATAAAAGGCCGATGCGGGCTTGCCCGGT
LopQuadripinnata     CTCCCGACTTCTGGAAGGGACGCATTTATTAGATAAAAGGCCGATGCGGGCTTGCCCGGT
AlsSmithii           CTCCCGACCTCTGGAAGGGACGCATTTATTAGATAAAAGGCCGATGCGGGCTTGCCCGGT
AlsDealbata          CTCCCGACCTCTGGAAGGGACGCATTTATTAGATAAAAGGCCGATGCGGGCTTGCCCGGT
SphMedullaris        CTCCCGACTTCTGGAAGGGACGCATTTATTAGATAAAAGGCCGATGCGGGCTTGCCCGGT
Adiantum             CTCCCGACTTCTGGAAGGGACGCATTTATTAGATAAAAGGCCGATGCGGGCTTGCCCGGT

                   241                                                  300
DicArborescens       AATGCGGTGAATCATGATAACTTCCCGAATCGCACGGCCTCGGCGCCGGCGATGCTTCAT
DicFibrosa           AATGCGGTGAATCATGATAACTTCCCGAATCGCACGGCCTCGGCGCCGGCGATGCTTCAT
DicLanata            AATGCGGTGAATCATGATAACTTCCCGAATCGCACGGCCTCGGCGCCGGCGATGCTTCAT
DicAntHybseq         AATGCGGTGAATCATGATAACTTCCCGAATCGCACGGCCTCGGCGCCGGCGATGCTTCAT
LopQuadripinnata     AATGCGGTGAATCATGATAACTTCCCGAATCGCACGGCCTCGGCGCCGGCGATGCTTCAT
AlsSmithii           ATTGCGGTGAATCATGATAACTTCCCGAATCGCACGGCCTCGGCGCCGGCGATGCTTCAT
AlsDealbata          ATTGCGGTGAATCATGATAACTTCCCGAATCGCACGGCCTCGGCGCCGGCGATGCTTCAT
SphMedullaris        AATGCGGTGAATCATGATAACTTCCCGAATCGCACGGCCTCGGCGCCGGCGATGCTTCAT
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Adiantum                ATTGCGGTGAATCATGATAACTTTCCGAATCGCACGGCCTTGGCGCCGGCGATGCTTCAT

                        301                                                   360
DicArborescens          TCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTGACGG
DicFibrosa              TCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTGACGG
DicLanata               TCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTGACGG
DicAntHybseq            TCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTGACGG
LopQuadripinnata        TCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTGACGG
AlsSmithii              TCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTGACGG
AlsDealbata             TCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTGACGG
SphMedullaris           TCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTGACGG
Adiantum                TCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTGACGG

                        361                                                   420
DicArborescens          GTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCA
DicFibrosa              GTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCA
DicLanata               GTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCA
DicAntHybseq            GTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCA
LopQuadripinnata        GTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCA
AlsSmithii              GTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCA
AlsDealbata             GTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCA
SphMedullaris           GTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCA
Adiantum                GTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCA

                        421                                                   480
DicArborescens          AGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAAT
DicFibrosa              AGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAAT
DicLanata               AGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAAT
DicAntHybseq            AGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAAT
LopQuadripinnata        AGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAAT
AlsSmithii              AGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAAT
AlsDealbata             AGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAAT
SphMedullaris           AGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAAT
Adiantum                AGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAAT

                        481                                                   540
DicArborescens          AACAATACTGGGCTTTTTTAAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAAC
DicFibrosa              AACAATACTGGGCTTTTTTAAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAAC
DicLanata               AACAATACTGGGCTTTTTTAAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAAC
DicAntHybseq            AACAATACTGGGCTTTTTTAAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAAC
LopQuadripinnata        AACAATACTGGGCTTTTTTAAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAAC
AlsSmithii              AACAATACTGGGCTTTTTTAAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAAC
AlsDealbata             AACAATACTGGGCTTTTTTAAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAAC
SphMedullaris           AACAATACTGGGCTTTTTTAAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAAC
Adiantum                AACAATACTGGGCTTTTTCAAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAAC

                        541                                                   600
DicArborescens          GAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGC
DicFibrosa              GAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGC
DicLanata               GAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGC
DicAntHybseq            GAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGNTAATTCCAGCTCCAATAGC
LopQuadripinnata        GAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGC
AlsSmithii              GAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGC
AlsDealbata             GAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGC
SphMedullaris           GAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGC
Adiantum                GAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGC

                        601                                                   660
DicArborescens          GTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGGCGGGGCGAGCGG
DicFibrosa              GTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGGCGGGGCGAGCGG
DicLanata               GTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGGCGGGGCGAGCGG
DicAntHybseq            GTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGGCGGGGCGAGCGG
LopQuadripinnata        GTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGGCGGGGCGAGCGG
AlsSmithii              GTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGGCGGGGCGAGCGG
AlsDealbata             GTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGGCGGGGCGAGCGG
SphMedullaris           GTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGGCGGGGCGAGCGG
Adiantum                GTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGGCGGGGCGAGCGG

                        661                                                   720
DicArborescens          TCCGCCTCTTCTGGTGTGCACTGGTCGCTCCGCCCTTTCTGTCGGGGACGCGCTCCTGGC
DicFibrosa              TCCGCCTCTTCTGGTGTGCACTGGTCGCTCCGCCCTTTCTGTCGGGGACGCGCTCCTGGC
DicLanata               TCCGCCTCTTCTGGTGTGCACTGGTCGCTCCGCCCTTTCTGTCGGGGACGCGCTCCTGGC
DicAntHybseq            TCCGCCTCTTCTGGTGTGCACTGGTCGCTCCGCCCTTTCTGTCGGGGACGCGCTCCTGGC
LopQuadripinnata        TCCGCCTCTTCTGGTGTGCACTGGTCGCTCCGCCCTTTCTGTCGGGGACGCGCTCCTGGC
AlsSmithii              TCCGCCTCTTCTGGTGTGCACTGGTCGCTCCGCCCTTTCTGTCGGGGACGCGCTCCTGGC
AlsDealbata             TCCGCCTCTTCTGGTGTGCACTGGTCGCTCCGCCCTTTCTGTCGGGGACGCGCTCCTGGC
SphMedullaris           TCCGCCTCTTTTGGTGTGCACTGGTCGCTCCGCCCTTTCTGTCGGGGACGCGCTCCTGGC
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```
Adiantum                TCCGCCTTCTTTGGTGTGCACTGGTCGCTCCGCCCTTTCTGTCGGGGACGCGCTCCTGGC

                        721                                                 780
DicArborescens           CTTAGCTGGCTGGGACGTGGATTCGGCGATGTTACTTTGAAAAAATTAGAGTGCTCAAAG
DicFibrosa               CTTAGTTGGCTGGGACGCGGATTCGGCGATGTTACTTTGAAAAAATTAGAGTGCTCAAAG
DicLanata                CTTAGCTGGCTGGGACGCGGATTCGGCGATGTTACTTTGAAAAAATTAGAGTGCTCAAAG
DicAntHybseq             CTTAGCTGGCTGGGACGCGGATTCGGCGATGTTACTTTGAAAAAATTAGAGTGCTCAAAG
LopQuadripinnata        CTTAGCTGGCTGGGACGCGGATTCGGCGATGTTACTTTGAAAAAATTAGAGTGCTCAAAG
AlsSmithii               CTTAGCTGGCTGGGACGCGGATTCGGCGATGTTACTTTGAAAAAATTAGAGTGCTCAAAG
AlsDealbata              CTTTGTTGGCTGGGACGCGGATTCGGCGATGTTACTTTGAAAAAATTAGAGTGCTCAAAG
SphMedullaris            CTTAGTTGGCTGGGACGCGGATTCGGCGATGTTACTTTGAAAAAATTAGAGTGCTCAAAG
Adiantum                 CTTAATTGGCTGGGACGCGGATTCGGCGATGTTACTTTGAAAAAATTAGAGTGCTCAAAG

                        781                                                 840
DicArborescens           CAAGCCTATGCTCTGAATACATTAGCATGGAATAACGCGATAGGACTCTGGTCCTATTGT
DicFibrosa               CAAGCCTATGCTCTGAATACATTAGCATGGAATAACGCGATAGGACTCTGGTCCTATTGT
DicLanata                CAAGCCTATGCTCTGAATACATTAGCATGGAATAACGCGATAGGACTCTGGTCCTATTGT
DicAntHybseq             CAAGCCTATGCTCTGAATACATTAGCATGGAATAACGCGATAGGACTCTGGTCCTATTGT
LopQuadripinnata        CAAGCCTATGCTCTGAATACATTAGCATGGAATAACGCGATAGGACTCTGGTCCTATTGT
AlsSmithii               CAAGCCTATGCTCTGAATACATTAGCATGGAATAACGCGATAGGACTCTGGTCTTATTGT
AlsDealbata              CAAGCCTATGCTCTGAATACATTAGCATGGAATAACGCGATAGGACTCTGGTCTTATTGT
SphMedullaris            CAAGCCTATGCTCTGAATACATTAGCATGGAATAACGCGATAGGACTCTGGTCTTATTGT
Adiantum                 CAAGCCTATGCTCTGAATACATTAGCATGGAATAACGCGATAGGACTCTGGTCCTATTGT

                        841                                                 900
DicArborescens           GTTGGTCTTCGGGACCGGAGTAATGATTAATAGGGACAGTTGGGGGCATTCGTATTTCAT
DicFibrosa               GTTGGTCTTCGGGACCGGAGTAATGATTAATAGGGACAGTTGGGGGCATTCGTATTTCAT
DicLanata                GTTGGTCTTCGGGACCGGAGTAATGATTAATAGGGACAGTTGGGGGCATTCGTATTTCAT
DicAntHybseq             GTTGGTCTTCGGGACCGGAGTAATGATTAATAGGGACAGTTGGGGGCATTCGTATTTCAT
LopQuadripinnata        GTTGGTCTTCGGGACCGGAGTAATGATTAATAGGGACGGTTGGGGGCATTCGTATTTCAT
AlsSmithii               GTTGGTCTTCGGGACCGGAGTAATGATTAATAGGGACGGTTGGGGGCATTCGTATTTCAT
AlsDealbata              GTTGGTCTTCGGGACCGGAGTAATGATTAATAGGGACGGTTGGGGGCATTCGTATTTCAT
SphMedullaris            GTTGGTCTTCGGGACCGGAGTAATGATTAATAGGGACGGTTGGGGGCATTCGTATTTCAT
Adiantum                 GTTGGTCTTCGGGACCGGAGTAATGATTAATAGGGACAGTTGGGGGCATTCGTATTTCAT

                        901                                                 960
DicArborescens           TGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAG
DicFibrosa               TGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAG
DicLanata                TGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAG
DicAntHybseq             TGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAG
LopQuadripinnata        TGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAG
AlsSmithii               TGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAG
AlsDealbata              TGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAG
SphMedullaris            TGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAG
Adiantum                 TGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAG

                        961                                                1020
DicArborescens           GATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATACCGTCCTA
DicFibrosa               GATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATACCGTCCTA
DicLanata                GATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATACCGTCCTA
DicAntHybseq             GATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATACCGTCCTA
LopQuadripinnata        GATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATACCGTCCTA
AlsSmithii               GATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATACCGTCCTA
AlsDealbata              GATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATACCGTCCTA
SphMedullaris            GATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATACCGTCCTA
Adiantum                 GATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATACCGTCCTA

                        1021                                               1080
DicArborescens           GTCTCAACCATAAACGATGCCGACTAGGGATTGGCGGATGTTACTTCGATGACTCCGCCA
DicFibrosa               GTCTCAACCATAAACGATGCCGACTAGGGATTGGCGGATGTTACTTCGATGACTCCGCCA
DicLanata                GTCTCAACCATAAACGATGCCGACTAGGGATTGGCGGATGTTACTTCGATGACTCCGCCA
DicAntHybseq             GTCTCAACCATAAACGATGCCGACTAGGGATTGGCGGATGTTACTTCGATGACTCCGCCA
LopQuadripinnata        GTCTCAACCATAAACGATGCCGACTAGGGATTGGCGGATGTTACTTCGATGACTCCGCCA
AlsSmithii               GTCTCAACCATAAACGATGCCGACTAGGGATTGGCGGATGTTACTTTGATGACTCCGCCA
AlsDealbata              GTCTCAACCATAAACGATGCCGACTAGGGATTGGCGGATGTTACTTTGATGACTCCGCCA
SphMedullaris            GTCTCAACCATAAACGATGCCGACTAGGGATTGGCGGATGTTACTTTGATGACTCCGCCA
Adiantum                 GTCTCAACCATAAACGATGCCGACTAGGGATTGGCGGATGTTACTTTGATGACTCCGCCA

                        1081                                               1140
DicArborescens           GCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCAAGGCTGAAA
DicFibrosa               GCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCAAGGCTGAAA
DicLanata                GCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCAAGGCTGAAA
DicAntHybseq             GCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCAAGGCTGAAA
LopQuadripinnata        GCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCAAGGCTGAAA
AlsSmithii               GCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCAAGGCTGAAA
AlsDealbata              GCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCAAGGCTGAAA
SphMedullaris            GCACCTTATGGGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCAAGGCTGAAA
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Adiantum                GCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAA

                        1141                                                1200
DicArborescens           CTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCA
DicFibrosa               CTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCA
DicLanata                CTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCA
DicAntHybseq             CTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCA
LopQuadripinnata        CTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCA
AlsSmithii               CTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCA
AlsDealbata              CTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCA
SphMedullaris            CTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCA
Adiantum                 CTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCA

                        1201                                                1260
DicArborescens           ACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCTCTTTCT
DicFibrosa               ACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCTCTTTCT
DicLanata                ACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCTCTTTCT
DicAntHybseq             ACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCTCTTTCT
LopQuadripinnata        ACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCTCTTTCT
AlsSmithii               ACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCTCTTTCT
AlsDealbata              ACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCTCTTTCT
SphMedullaris            ACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCTCTTTCT
Adiantum                 ACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCTCTTTCT

                        1261                                                1320
DicArborescens           TGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAA
DicFibrosa               TGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAA
DicLanata                TGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAA
DicAntHybseq             TGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAA
LopQuadripinnata        TGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAA
AlsSmithii               TGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAA
AlsDealbata              TGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAA
SphMedullaris            TGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAA
Adiantum                 TGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAA

                        1321                                                1380
DicArborescens           TTCCGTTAACGAACGAGACCTCAGCCTGCTAACTAGTTACACGAAGGATCCTCTTCGTGG
DicFibrosa               TTCCGTTAACGAACGAGACCTCAGCCTGCTAACTAGTTACACGAAGGATCCTCTTCGTGG
DicLanata                TTCCGTTAACGAACGAGACCTCAGCCTGCTAACTAGTTACACGAAGGATCCTCTTCGTGG
DicAntHybseq             TTCCGTTAACGAACGAGACCTCAGCCTGCTAACTAGTTACACGAAGGATCCTCTTCGTGG
LopQuadripinnata        TTCCGTTAACGAACGAGACCTCAGCCTGCTAACTAGTTACACGAAGGATCCTCTTCGTGG
AlsSmithii               TTCCGTTAACGAACGAGACCTCAGCCTGCTAACTAGTTACACGAAGGATCTCCTTCGTGG
AlsDealbata              TTCCGTTAACGAACGAGACCTCAACCTGCTAACTAGTTACACGAAGGATCTTCTTCGTGG
SphMedullaris            TTCCGTTAACGAACGAGACCTCAGCCTGCTAACTAGTTACACGAAGGATCTCCTTCGTGG
Adiantum                 TTCCGTTAACGAACGAGACCTCAGCCTGCTAACTAGTTACACGAAGGATCCTCTTCGTGG

                        1381                                                1440
DicArborescens           CCAACTTCTTAGAGGGACTATGGCCGTCTAGGCCATGGAAGTTTGAGGCAATAACAGGTC
DicFibrosa               CCAACTTCTTAGAGGGACTATGGCCGTCTAGGCCATGGAAGTTTGAGGCAATAACAGGTC
DicLanata                CCAACTTCTTAGAGGGACTATGGCCGTCTAGGCCATGGAAGTTTGAGGCAATAACAGGTC
DicAntHybseq             CCAACTTCTTAGAGGGACTATGGCCGTCTAGGCCATGGAAGTTTGAGGCAATAACAGGTC
LopQuadripinnata        CCAACTTCTTAGAGGGACTATGGCCGTCTAGGCCATGGAAGTTTGAGGCAATAACAGGTC
AlsSmithii               CCAACTTCTTAGAGGGACTATGGCCGTCTAGGCCATGGAAGTTTGAGGCAATAACAGGTC
AlsDealbata              CCAACTTCTTAGAGGGACTATGGCCGTCTAGGCCATGGAAGTTTGAGGCAATAACAGGTC
SphMedullaris            CCAACTTCTTAGAGGGACTATGGCCGTCTAGGCCATGGAAGTTTGAGGCAATAACAGGTC
Adiantum                 CCAACTTCTTAGAGGGACTATGGCCGTCTAGGCCATGGAAGTTTGAGGCAATAACAGGTC

                        1441                                                1500
DicArborescens           TGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGAATTCAACGAGTTT
DicFibrosa               TGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGAATTCAACGAGTTT
DicLanata                TGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGAATTCAACGAGTTT
DicAntHybseq             TGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGAATTCAACGAGTTT
LopQuadripinnata        TGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGAATTCAACGAGTTT
AlsSmithii               TGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGAATTCAACGAGTTT
AlsDealbata              TGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGAATTCAACGAGTTT
SphMedullaris            TGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGAATTCAACGAGTTT
Adiantum                 TGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGAATTCAACGAGTTT

                        1501                                                1560
DicArborescens           ACCACCTGGGCCGATAGGCCCGGGTAATCTTTTGAAATTTCATCGTGATGGGGATAGATC
DicFibrosa               ACCACCTGGGCCGATAGGCCCGGGTAATCTTTTGAAATTTCATCGTGATGGGGATAGATC
DicLanata                ACCACCTGGGCCGATAGGCCCGGGTAATCTTTTGAAATTTCATCGTGATGGGGATAGATC
DicAntHybseq             ACCACCTGGGCCGATAGGCCCGGGTAATCTTTTGAAATTTCATCGTGATGGGGATAGATC
LopQuadripinnata        ACCACCTGGGCCGATAGGCCCGGGTAATCTTTTGAAATTTCATCGTGATGGGGATAGATC
AlsSmithii               ACCACCTGGGCCGATAGGCCCGGGTAATCTTTTGAAATTTCATCGTGATGGGGATAGATC
AlsDealbata              ACCACCTGGGCCGATAGGCCCGGGTAATCTTTTGAAATTTCATCGTGATGGGGATAGATC
SphMedullaris            ACCACCTGGGCCGATAGGCCCGGGTAATCTTTTGAAATTTCATCGTGATGGGGATAGATC
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Adiantum                ACCACCTGGGCCGACAGGCCCGGGTAATCTTTTGAAATTTCATCGTGATGGGGATAGATC

                        1561                                                1620
DicArborescens           ATTGCAATTATTGATCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT
DicFibrosa               ATTGCAATTATTGATCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT
DicLanata                ATTGCAATTATTGATCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT
DicAntHybseq             ATTGCAATTATTGATCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT
LopQuadripinnata        ATTGCAATTATTGATCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT
AlsSmithii               ATTGCAATTATTGATCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT
AlsDealbata              ATTGCAATTATTGATCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT
SphMedullaris            ATTGCAATTATTGATCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT
Adiantum                 ATTGCAATTATTGATCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT

                        1621                                                1680
DicArborescens           GACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAATGGTCCGGT
DicFibrosa               GACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAATGGTCCGGT
DicLanata                GACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAATGGTCCGGT
DicAntHybseq             GACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAATGGTCCGGT
LopQuadripinnata        GACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAATGGTCCGGT
AlsSmithii               GACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAATGGTCCGGT
AlsDealbata              GACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAATGGTCCGGT
SphMedullaris            GACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAATGGTCCGGT
Adiantum                 GACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAATGGTCCGGT

                        1681                                                1740
DicArborescens           GAAGTTTTCGGATCGCGGCGACGCTGGCGGTTCGCCGCCGGTGACGTTGTGAGAAGTTCA
DicFibrosa               GAAGTTTTCGGATCGCGGCGACGCTGGCGGTTCGCCGCCGGTGACGTTGTGAGAAGTTCA
DicLanata                GAAGTTTTCGGATCGCGGCGACGCTGGCGGTTCGCCGCCGGTGACGTTGTGAGAAGTTCA
DicAntHybseq             GAAGTTTTCGGATCGCGGCGACGCTGGCGGTTCGCCGCCGGTGACGTTGTGAGAAGTTCA
LopQuadripinnata        GAAGTTTTCGGATCGCGGCGACGCTGGCGGTTCGCCGCCGGTGACGTTGTGAGAAGTTCA
AlsSmithii               GAAGTTTTCGGATCGCGGCGACGCTGGCGGTTCGCTGCCGGTGACGTTGTGAGAAGTTCA
AlsDealbata              GAAGTTTTCGGATCGCGGCGACGCTGGCGGTTCGCTGCCGGTGACGTTGTGAGAAGTTCA
SphMedullaris            GAAGTTTTCGGATCGCGGCGACGCTGGCGGTTCGCTGCCGGTGAC----------------
Adiantum                 GAAGTTTTCGGATCGCGGCGACGCTGGCGGTTCGCCGCCGGTGACGTTGTGAGAAGTTCA

                        1741                                                1800
DicArborescens           TTAAGCCTTATCATTTAGAGGAAGG-----------------------------------
DicFibrosa               TTAAGCCTTATC-------------------------------------------------
DicLanata                TTAAGCCTTATCATTTAGA-----------------------------------------
DicAntHybseq             TTAAGCCTTATCATTTAGAGGAAG------------------------------------
LopQuadripinnata        TTAAGCCTTATCATTTAGAGGAAGGAGA--------------------------------
AlsSmithii               TTAAGCCTTATCATTTA-------------------------------------------
AlsDealbata              TTAAGCCTTATCATTTAGAGGAAGGAGAA------------------------------
SphMedullaris            -----------------------------------------------------------
Adiantum                 TTAAGCCTTATCATTTAGAGGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGC

                        1801   1811
DicArborescens           -----------
DicFibrosa               -----------
DicLanata                -----------
DicAntHybseq             -----------
LopQuadripinnata        -----------
AlsSmithii               -----------
AlsDealbata              -----------
SphMedullaris            -----------
Adiantum                 AGAAGGATCAA
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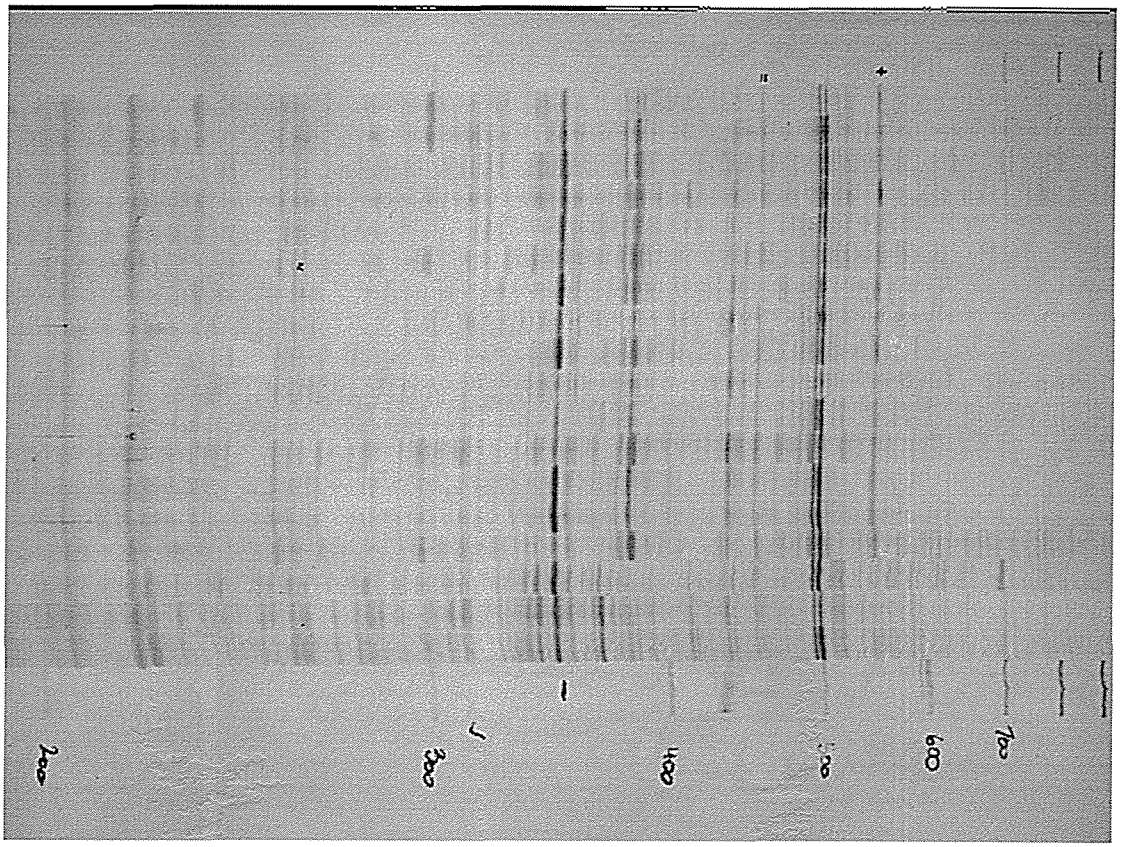
4.2 AFLP Gel Photos

Mse-CTG/Eco-ATA



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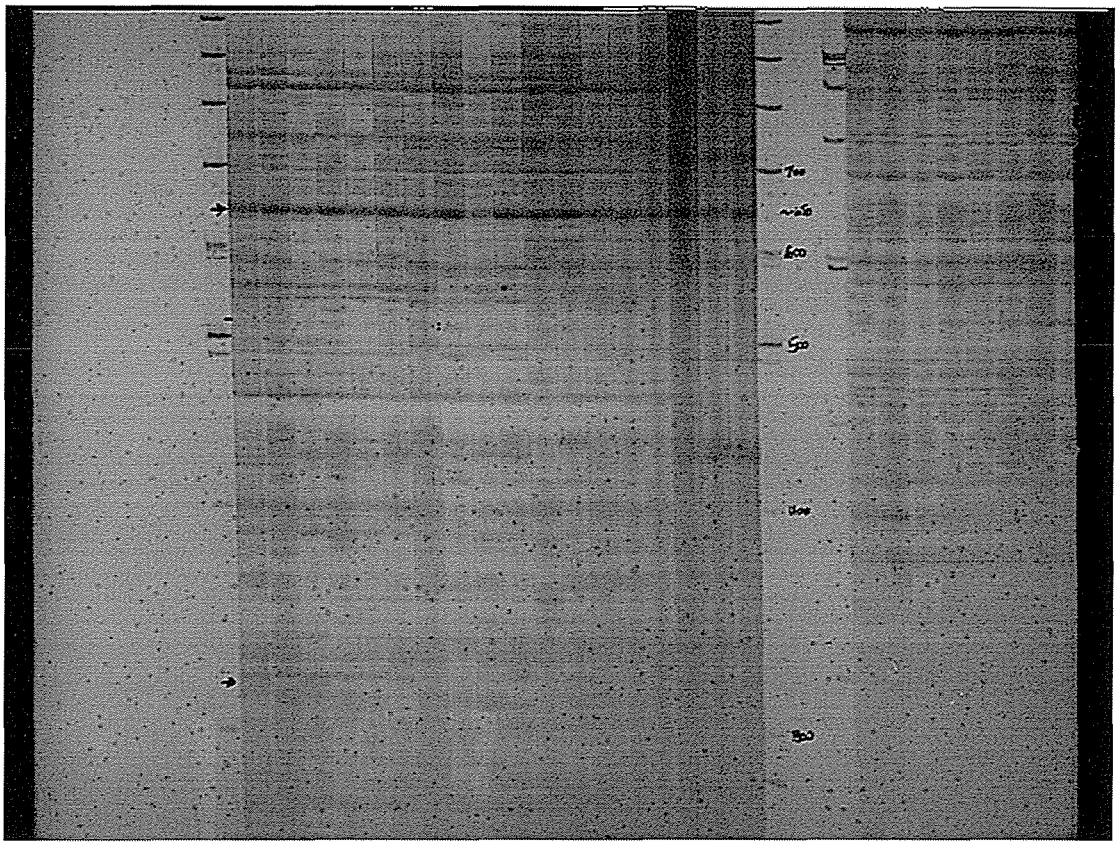

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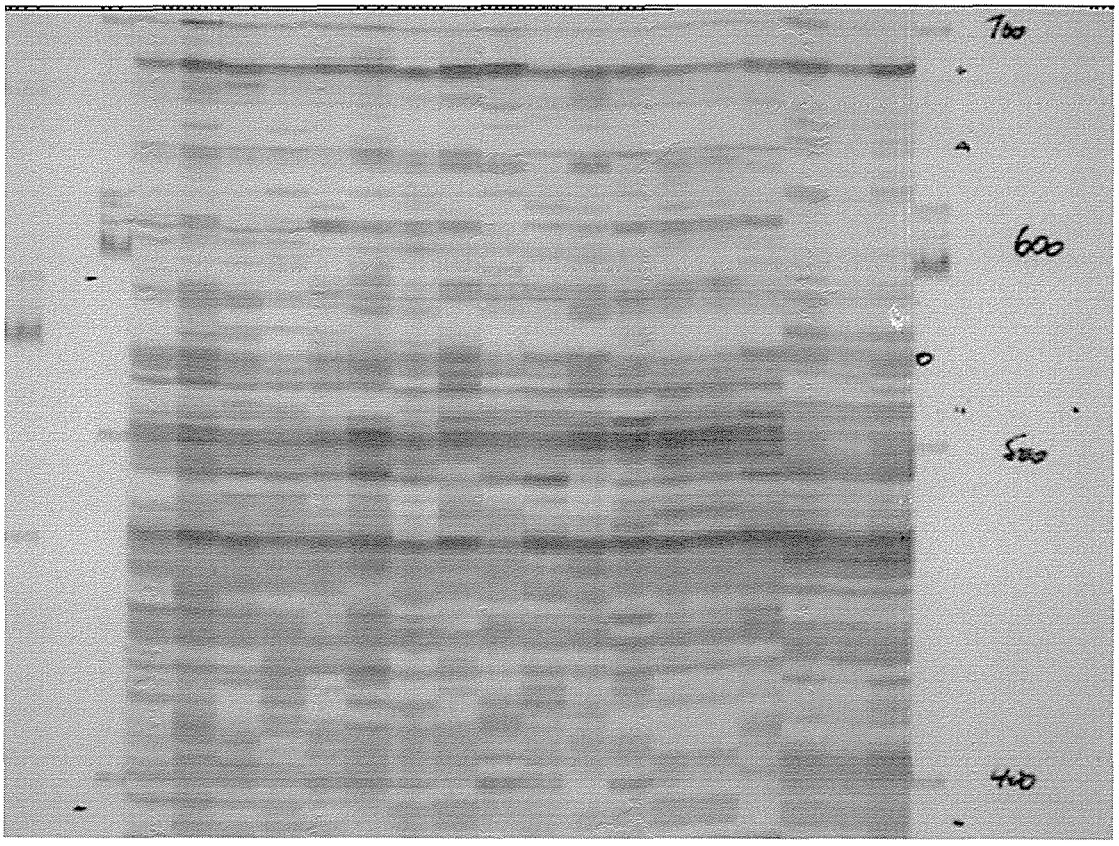

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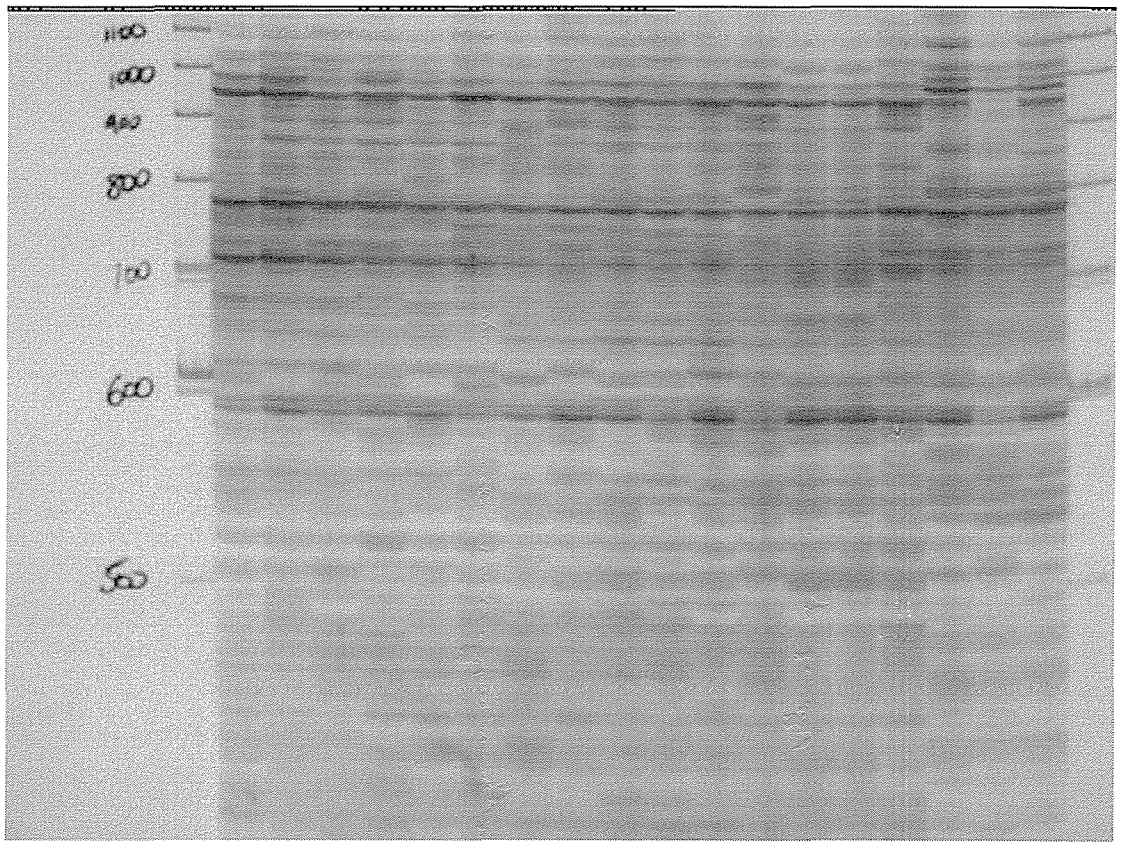

APPENDIX 5

5.1 DNA sequence alignments

5.1.1 Cyatheaceae *trnL-trnF* spacer

Sequence Label	Species	Source/GenBank Accession
AlCun	*Alsophila cunninghamii*	New Zealand/Auckland University
AlKer	*Alsophila kermadecensis*	New Zealand/Auckland University
AlCol	*Alsophila colensoi*	New Zealand/R.L. Lewis, M.F. Large
AlSmi	*Alsophila smithii*	New Zealand/M.F. Large
AlTri	*Alsophila tricolor*	New Zealand/R.L. Lewis, M.F. Large
AlMil	*Alsophila milnei*	New Zealand/Auckland University
SpMed2	*Sphaeropteris medullaris*	New Zealand/R.L. Lewis
SpRob	*Sphaeropteris robusta*	Lord Howe Island/RBG Sydney
SpBro	*Sphaeropteris excelsa*	Norfolk Island/RBG Sydney
SpTom	*Sphaeropteris tomentosissima*	New Guinea/S. Van der Mast
CyDel	*Sphaeropteris* sp. ? (as *C. delgadii*)	?/S. Van der Mast

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                1                                                          60
AlCun             ------------------TTGCCAATCT--------------ATATGTAGATTGTTCGGG
AlKer             ------------------------------------------------------TCGGG
AlCol             CAACAGAACAGGGTAGAATTGCCAATCT--------------ATATGTAGATTGTTCGGG
AlSmi             CAACAGAACAGGGTAGAATTGCCAATCT--------------ATATGTAGATTGTTCGGG
AlTri             CAAAAGAAAGGGGGAGAATTGCCAATCT--------------ATATGTAGATTGTTCGGG
AlMil             ----------------------C-ATCT--------------ATATGTAGATTGTTCGGG
SpMed2            CAACAGAACAGGGTAGAATTGCCAATCTTTATATATAGCTATATATATAGGCTGTTCGGA
SpRob             CAACAGAACAGGGTAGAATTGTCAATCTTTATATATAGCTATATATATAGGTTGTTCGTA
SpBro             CAACAGAACAGGGTAGAATTGTCAATCTTTATATATAGCTATATATATAGGTTGTTCGTA
SpTom             CAACAGAACAGGGTAGAATTGTCAATCTTTATATATAGCTATATATATAGGTTGTTCGTA
CyDel             CAACAGAACAGGGTAGAATTGTCAATCTTTATATATAGCTATATATATAGGTTGTTCGTA

               61                                                         120
AlCun             TTCATATGGTTATCTCAACCCCCCCCCCCCCCAAAAAAAAAAAAAACTATTGGGGGTGGGAG
AlKer             TTCATATGGTTATCTCAACCCCCCCCCCCCCCC-AAAAAAAAAAACTATTGGGGGTGGGAG
AlCol             TTCATATGGTTATCTCACCCCCCCCC----------AAAAAAACTATTGGGGGTGGGAG
AlSmi             TTCATATGGTTATCTCACCCCCCC----------------AAAACTATTGGGGGTGGGAG
AlTri             TTCATATGGTTATATCACCCCCTCC----------------AAACTATTGAGGG---GAG
AlMil             TTCATATGGTTATATCACCCCCTCC----------------AAACTATTGAGGG---GAG
SpMed2            TTC----GGTTATCTTACCCCTTCC----------------AAACTATTTGAAG---GAA
SpRob             TTCATATGGTTATCTTACCCCTTCC----------------AAACTAT------------
SpBro             TTCATATGGTTATCTTACCCCTTCC----------------AAACTAT------------
SpTom             TTCATATGGTTATCTTACCCCTTCC----------------AAACTAT------------
CyDel             TTCATATGGTTATCTTACCCCTTCC----------------AAACTAT------------

               121                                                        180
AlCun             TAGTAAATCTTAGATCTTTGACTCAGAGCTCGATAAATTTAGCTTAATACCCTGTCCTTT
AlKer             TAGTAAATCTTAGATCTTTGACTCAGAGCTCGATAAATTTAGCTTAATACCCTGTCCTTT
AlCol             TAGTAAATCTTAGATCTTTGACTCAGAGCTCGATAAATTTAGCTTAATACCCTGTCCTTT
AlSmi             TAGTAAATCTTAGATCTTTGACTCAGAGCTCGATAAATTTAGCTTAATACCCTGTCCTTT
AlTri             TAGTAAATCTTAGATCTTTGACTCAGAGCTCGATAAATTTAGCTTAATACCCTGTCCTTT
AlMil             TAGTAAATCTTAGATCTTTGACTCAGAGCTCGATAAATTTAGCTTAATACCCTGTCCTTT
SpMed2            TAGTAAATCTTAGATCTTTGACTCAGAGCTCGATAAATTTAGCTTAATACCCTGTCCTTT
SpRob             ----------------------------------AAATTTAGCTTAATACCCTGTCCTTT
SpBro             ----------------------------------AAATTTAGCTTAATACCCTGTCCTTT
SpTom             ----------------------------------AAATTTAGCTTAATACCCTGTCCTTT
CyDel             ----------------------------------AAATTTAGCTTAATACCCTGTCCTTT

               181                                                        240
AlCun             CGACTTTAACCTTTTATTTGAGTATGGATCACAAATGAACCACTGAGCAAGCCTAAAAGA
AlKer             CGACTTTAACCTTTTATTTGAGTATGGATCACAAATGAACCACTGAGCAAGCCTAAAAGA
AlCol             CGACTTTAACCTTTTATTTGAGTATGGATCACAAATGAACCACCGAGCAAGCCTAAAAGA
AlSmi             CGACTTTAACCTTTTATTTGAGTATGGATCACAAATGAACCACTGAGCAAGCCTAAAAGG
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AlTri          CGACTTTAACCTTTTATTTGAGTATGGATCACAAATGAACCACTGAGCAAGCCTAAAAGA
AlMil          CGACTTTAACCTTTTATTTGAGTATGGATCACAAATGAACCACTGAGCAAGCCTAAAAGA
SpMed2         TGACTTTAACCTTTTATTTGAGTATGGATCACAAATGAACCACTGAGCAAGCCTAAAGGG
SpRob          TGACT----------ATTTGAGTATGGATCACAAATGAACCACTGAGCAAGCCTAAAGGG
SpBro          TGACT----------ATTTGAGTATGGATCACAAATGAACCACTGAGCAAGCCTAAAGGG
SpTom          TGACT----------ATTTGAGTATGGATCACAAATGAACCACTGAGCAAGCCTAAAGGG
CyDel          TGACT----------ATTTGAGTATGGATCACAAATGAACCACTGAGCAAGCCTAAAGGG

               241                                                       300
AlCun          CTTTTTGAGTGGTTCATTTGAAAACTATAGAAAATATTAAACAAACGACT----------
AlKer          CTTTTTGAGTGGTTCATTTGAAAACTATAGAAAATATTAAACAAACGACT----------
AlCol          CTTTTTGAGTGGTTCATTTGAAAACTATAGAAAATATTAAACAAACGGCT----------
AlSmi          CTTTTTGAGTGGTTCATTTGAAAACTATAGAAAATAGGAAACAAACGACT----------
AlTri          CTTTTTGAGTGGTTCATTTGAAAACTATAGAAAATATTAAACAAACGACTATTTTTTTTT
AlMil          CTTTTTGAGTGGTTCATTTGAAAACTATAGAAAATATTAAACAAACGACTATTTTTTTTT
SpMed2         CTTTTTGAGTGGTTCATTTGAAAACTATAGAAAATCTTAAACAAACGACTA---------
SpRob          CTTTTTGAGTGGTTCATTTGAAAACTATAGAAAATCTTAAACAAACGACTA---------
SpBro          CTTTTTGAGTGGTTCATTTGAAAACTATAGAAAATCTTAAACAAACGACTA---------
SpTom          CTTTTTGAGTGGTTCATTTGAAAACTATAGAAAATCTTAAACAAACGACTA---------
CyDel          CTTTTTGAGTGGTTCATTTGAAAACTATAGAAAATCTTAAACAAACGACTA---------

               301                                                       360
AlCun          -----CTTTTTTACCGAGGCTATTGGTTTGATTGAGCAGAGATAGATCGAATAAAAAAGA
AlKer          -----CTTTTTTACCGAGGCTATTGGTTTGATTGAGCAGAGATAGATCGAATAAAAAAGA
AlCol          -----CTTTTTTACCGAGGCTATTGGTTTGATTGAGCAGAGATAGATCGAATAAAAAAGA
AlSmi          -----CTTTTTTACCGAGGCTATTGGTTTGATTGAGCAGAGATAGATCGAATAAAAAAGA
AlTri          TT---TTTTTTTACCGAGGCTATTGGTTTGATTGAGCAGAGATAGATCGAATAAAAAAGA
AlMil          TTTTTTTTTTTTACCGAGGCTATTGGTTTGATTGAGCAGAGATAGATCGAATAAAAAAGA
SpMed2         -----TTGTTTTACCGAGGTTATTGATTTGATTGAGCAGAGATAGATCGAATAAAAAAGA
SpRob          -----TTGTTTTACCGAGGTTATTGGTTTGATTGAGCAGAGATAGATCGAA-------GA
SpBro          -----TTGTTTTACCGAGGTTATTGGTTTGATTGAGCAGAGATAGATCGAA-------GA
SpTom          -----TTGTTTTACCGAGGTTATTGGTTTGATTGAGCAGAGATAGATCGAA-------GA
CyDel          -----TTGTTTTACCGAGGTTATTGGTTTGATTGAGCAGAGATAGATCGAA-------GA

               361                                                       420
AlCun          TATCTGTCTCATTTGGATTTGAGGCATCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
AlKer          TATCTGTCTCATTTGGATTTGAGGCATCTCCG-AGAGACGTTG------------------
AlCol          TATCTGTCTCATTTGGATTTGAGGCATCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
AlSmi          TATCTGTCTCATTTGGATTTGAGGCATCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
AlTri          TATCTGTCTCATTTGGATTTGAGGCATCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
AlMil          TATCTGTCTCATTTGGATTTGAGGCATCTCCGTAGAGACGTTGGCCGGGATAGCTCAGCC
SpMed2         TATCTGTCTCATTTGGATTTGAGGCATCTCCGTAGAGACGTTGGCCGGGATAGCTCAGTC
SpRob          TATCTGTCTCATTTGGATTTGAGGCATCTCCGTAGAGACGTTGGCCGGGATAGCTCAGTC
SpBro          TATCTGTCTCATTTGGATTTGAGGC-----CGTAGAGACGTTGGCCGGGATAGCTCAGTC
SpTom          TATCTGTCTCATTTGGATTTGAGGC-----CGTAGAGACGTTGGCCGGGATAGCTCAGTC
CyDel          TATCTGTCTCATTTGGATTTGAGGC-----CGTAGAGACGTTGGCCGGGATAGCTCAATC

               420                 443
AlCun          GGTAGAGCA----------
AlKer          ----------
AlCol          GGTAGAGCAGAGGACTGAAAATC
AlSmi          GGTAGAGCAGAGGACTGAAAATC
AlTri          GGTAGAGCAAAGAACTGAAAATC
AlMil          GGTA----------
SpMed2         GGTAGAGCAGAGGACTGAAAATC
SpRob          GGTAGAGCAGAGGACTGAAAATC
SpBro          GGTAGAGCAGAGGACTGAAAATC
SpTom          GGTAGAGCAGAGGACTGAAAATC
CyDel          GGTAGAGCAAANGACTGAAAATC
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