## Re-evaluation of the taxonomic status of Cyathea kermadecensis and C. milnei (Cyatheaceae) supports their continued recognition

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ABSTRACT: Two species of *Cyathea* (Cyatheaceae) have been recognised as endemic to Raoul Island in the Kermadec Islands: *C. kermadecensis* and *C. milnei*. However, their relationships to their mainland relatives – *C. cunninghamii* and *C. dealbata*, respectively – have been uncertain, with their morphological distinctiveness in question. Here, we review their taxonomic status. The DNA sequences investigated are uninformative as to delimitation of the Kermadec plants, but they do support a close relationship to the mainland species. Morphologically, we find that *C. kermadecensis* can be consistently distinguished from *C. cunninghamii*, and likewise *C. milnei* from *C. dealbata*. With no data to reassess whether species or subspecies rank is most appropriate, we recommend the taxonomically conservative approach of retaining both *C. kermadecensis* and *C. milnei* as separate species for now.

KEYWORDS: Cyatheaceae, Cyathea cunninghamii, Cyathea dealbata, Cyathea milnei, Cyathea kermadecensis, ferns, Kermadec Islands, New Zealand Botanical Region, taxonomy.

### Introduction

Seven species in the tree fern family Cyatheaceae are currently recognised as indigenous to the New Zealand Botanical Region (sensu Allan 1961) – Cyathea colensoi (Hook.f.) Domin, C. cunninghamii Hook.f., C. dealbata (G.Forst.) Sw., C. kermadecensis W.R.B.Oliv., C. medullaris (G.Forst.) Sw., C. milnei Hook. ex Hook.f. and C. smithii Hook.f. – along with one fully naturalised species, Cyathea cooperi (Hook. ex F.Muell.) Domin (Brownsey et al. 1985; Brownsey & Smith-Dodsworth 2000). Of the indigenous species, five occur on the main islands of New Zealand, and two, C. kermadecensis and C. milnei, are endemic to Raoul Island. This is in the Kermadec Islands, c. 980 km northeast of New Zealand's North Island (Sykes 1977). These species from the Kermadec Islands, particularly their taxonomic status, are the focus of this paper.

Cyathea milnei was first collected during the voyage of HMS Herald in 1854. Joseph Hooker (1856) used plant

collections made by John Macgillivray and William Milne to publish an account of the botany of the Kermadec Islands, but initially misidentified the tree ferns as C. medullaris. He only later recognised C. milnei as a new species (Hooker 1867), based on a brief manuscript description by William Hooker. Nevertheless, he asserted that the species was 'very similar to C. medullaris', a belief that was held by Cheeseman (1888, 1906), Oliver (1910), Dobbie (1921) and Crookes (1963). Cheeseman (1925) and Allan (1961) were non-committal about its affinities, and it was not until Holttum (1964) revised Cyathea in Australasia and the Pacific that its true affinity became clear. Holttum noted that it was 'very near C. dealbata, the only clear distinction ... being the lack of white covering on lower surface of lamina' and 'the indusia are perhaps more fragile than in C. dealbata and do not so persistently form cups with [an] entire rim'. This affinity was also noted by Sykes (1977) and Brownsey & Smith-Dodsworth (2000). Recently, the status of *C. milnei* as a distinct species has been questioned by Dawson & Lucas (2011), while de Lange (2009a) has suggested that plants from the far north of New Zealand around Te Paki are very similar to *C. milnei*. We have also noted this during fieldwork in Northland, and have speculated that the morphological characters used to separate *C. milnei* may actually be encompassed by the variation exhibited by *C. dealbata*.

Cyathea kermadecensis was not recorded until W.R.B. Oliver's 10-month visit to the Kermadec Islands in 1908. In his account of the vegetation, Oliver (1910) pointed out that only one species had been recognised from Sunday [Raoul] Island, but that two species had been confused under the name C. milnei. In describing C. kermadecensis he identified a number of morphological characters that distinguished them, and noted that they were also ecologically distinct, C. kermadecensis being more common in the higher, wetter forest, and C. milnei being more common in dry forest at lower altitudes. Cheeseman (1925) acknowledged his confusion of the two species and accepted that both occurred on Raoul. Subsequently, Allan (1961), Crookes (1963) and Holttum (1964) all accepted C. kermadecensis as a distinct species. Sykes (1977), Brownsey & Smith-Dodsworth (2000), de Lange (2009b) and Dawson & Lucas (2011) also all accepted the species, but pointed out that it was very similar to C. cunninghamii.

The status of Cyathea cunninghamii itself has not been universally agreed by New Zealand authors. It was described by Joseph Hooker (in W.J. Hooker 1854) but he later observed that it was 'very similar to C. medullaris, and perhaps only a variety of it' (Hooker 1867). Thomson (1882) agreed, noting that 'probably it ought to be reduced to the rank of variety of C. medullaris'. Cheeseman (1906, 1925) and Dobbie (1921) regarded it as a separate species, but continued to ally it with C. medullaris. However, Allan (1961) noted that 'the status of the various forms that have been assigned to C. cunninghamii needs much further study, including the possibility that some may be the progeny of C. medullaris × C. smithii'. Crookes (1963) accepted the species and allied it with C. medullaris, but concluded that 'the species needs further study'. Holttum (1964) finally demonstrated that C. cunninghamii was a distinct species, indicating that it was fundamentally different to C. medullaris by placing the two in different subgenera. Brownsey (1979) confirmed this distinction, showing that it was actually closer to C. smithii, and provided illustrations of the scales and indusia to distinguish all three species.

In preparing the treatment of Cyatheaceae for the electronic *Flora of New Zealand* (Brownsey & Perrie 2015a), including typification (Brownsey & Perrie 2015b), we have examined all the New Zealand species in detail. We present here the results of our comparisons of the Kermadec Islands species with their mainland relatives. We address concerns about their distinctiveness, and provide more detail than Holttum (1964), the only previous critical comparison.

### Methods

The collections of New Zealand *Cyathea* in AK, CHR and WELT were examined (herbarium abbreviations follow Thiers 2015). We also inspected mature plants of *C. kermadecensis* and *C. milnei* in cultivation at Otari-Wilton's Bush, Wellington. This was combined with previously published information about morphology and ecology. Because it is closely related (Korall *et al.* 2007), we included the Australian *C. australis* in our comparison of *C. milnei* with *C. dealbata*.

Additionally, DNA sequences for the rbcL and trnL-trnF locus (trnL intron, trnL 3'-exon and the trnL-trnF intergenic spacer) were investigated because they are available for many Cyathea species, and because we have found them (particularly the trnL-trnF locus) to be useful for discerning closely related fern species (e.g. Shepherd et al. 2007; Perrie et al. 2013, 2014). Sequences for C. kermadecensis and C. milnei were generated for individuals cultivated at Otari-Wilton's Bush. These were vouchered with WELT P027384 and P027383, respectively. Extraction of genomic DNA from silica gel-dried frond tissue, polymerase chain reaction (PCR) amplification of the target loci, purification of PCR products and DNA sequencing followed Shepherd et al. (2007), but the rbcL sequences were amplified using the primers ESRBCL1F and ESRBCL1361 of Schuettpelz & Pryer (2007). GenBank accession numbers are given in Table 1.

The sequences for *Cyathea kermadecensis* were compared with sequences previously published to GenBank for *C. cunninghamii*, along with other close relatives as indicated by previous studies (e.g. Janssen *et al.* 2008; Korall & Pryer 2014). The same was done for *C. milnei* and *C. dealbata*. All the sequences compared are noted in Table 1. Sequences were aligned using Clustal X v. 2.1 (Larkin *et al.* 2007). Because of the small number of genetic differences recovered among the focal species, we did not undertake phylogenetic analyses.

Species	rbcL GenBank accession	trnL-trnF GenBank accession	Reference
C. kermadecensis	KR153993	KR153995	New to this study
C. colensoi	AM177322	AM410318	Korall <i>et al.</i> (2007)
C. cunninghamii	AM410211	AM410339	Korall <i>et al.</i> (2007)
C. smithii	AM410210	AM410338	Korall <i>et al.</i> (2007)
C. milnei	KR153992	KR153994	New to this study
C. australis	AM177319	AM410314	Korall <i>et al.</i> (2007)
C. dealbata	AM410199	AM410326	Korall <i>et al.</i> (2007)
C. macarthurii	AM410204	AM410335	Korall <i>et al.</i> (2007)

Table 1 Cyathea samples included in the DNA sequence comparisons of C. kermadecensis and C. milnei.

### Results

# Morphology of *Cyathea cunninghamii* and *C. kermadecensis*

Cyathea cunninghamii and C. kermadecensis both belong to subgenus Alsophila (Korall et al. 2007), lacking the scales with dark marginal setae found in subgenus Sphaeropteris (Brownsey 1979: fig. 1H). They differ from all other New Zealand species of Cyathea in having indusia that open at maturity to form a hood shape (Brownsey 1979: fig. 2B), and a more diverse array of hairs and scales, including larger, pale scales with a bullate base and a single apical seta (Brownsey 1979: fig. 1D), and acaroid (or stellate) scales that sometimes have expanded bases (Brownsey 1979: figs. 1E-G; Brownsey & Smith-Dodsworth 2000: fig. 102). Both taxa grow into tree ferns with trunks up to 20 m tall, covered in appressed stipe bases or hexagonal scars, and bear fronds that drop with age (Oliver 1910: pl. XXII; Large & Braggins 2004: pls 39-40; Dawson & Lucas 2011: 110-111). The fronds themselves are of very similar proportions and dissection (Table 2), and have stipe bases that are tuberculate and rough to the touch (Large & Braggins 2004: pl. 55). Ecologically, C. cunninghamii and C. kermadecensis are also similar, occurring as emergent species in forest in wetter areas.

One of the most obvious differences between the two species (Table 3) is that *Cyathea kermadecensis* lacks the thickened red acaroid scales that are common in *C. cunninghamii* 

(Brownsey 1979: fig. 1E). However, C. kermadecensis does have colourless acaroid scales (Brownsey 1979: fig. 1F), sometimes forming a dense appressed tomentum on the stipe, rachis and costae. In C. cunninghamii, both red and colourless acaroid scales are often present, sometimes also with expanded pale bases (Brownsey 1979: fig. 1G), but in C. kermadecensis the scales with expanded bases normally have only colourless apical proliferations, not thickened red ones. Cyathea kermadecensis also usually has irregularly curled acicular hairs on the abaxial surfaces (absent in C. cunninghamii), and a greater proportion of larger, pale scales with bullate bases (Brownsey 1979: fig. 1D) that tend to obscure the acaroid scales (Fig. 1). The tertiary pinnae of C. kermadecensis are usually crenate rather than deeply divided. The stipe bases are predominantly black with pale brown scales in C. cunninghamii, whereas both are pale or red-brown in C. kermadecensis (Figs 2 and 3).

# Morphology of *Cyathea dealbata* and *C. milnei*

Cyathea milnei and C. dealbata also belong to subgenus Alsophila, and lack the scales with dark marginal setae characteristic of subgenus Sphaeropteris. They differ from all other New Zealand species of Cyathea in having indusia that open at maturity to form a deep cup, and having curled hairs, rather than scales, as the predominant indumentum on the abaxial lamina surfaces (Brownsey & Smith-

Table 2 Trunk and frond dimensions for Cyathea cunninghamii and C. kermadecensis, as well as C. dealbata and C. milnei.

	C. cunninghamii	C. kermadecensis	C. dealbata	C. milnei
Trunk height (m)	<20	<20	<12	<8
Frond length (mm)	1500-3000	2250-4000	2000-4000	1500-4000
Stipe length (mm)	80-450	80-250	80-900	70-400
Stipe scale length (mm)	<50	<35	<70	<50
Stipe scale width (mm)	1–2	1	<3	<3
Lamina dissection	2-pinnnate-pinnatifid to 3-pinnate-pinnatifid	2-pinnnate-pinnatifid to 2-pinnate-pinnatisect	2-pinnnate-pinnatifid to 2-pinnate-pinnatisect	2-pinnnate-pinnatifid to 2-pinnate-pinnatisect
Length of longest primary pinna (mm)	270-600	325–610	290-650	350–700
Width of longest primary pinna (mm)	80-210	110–195	135–240	150–260
Length of longest secondary pinna (mm)	43–110	65–115	70–145	85–145
Width of longest secondary pinna (mm)	9–28	14–35	13-30	15–27
Length of longest tertiary pinna (mm)	5–15	8–22	7–18	8–15
Width of longest tertiary pinna (mm)	1.5–3	2–2.5	2-4	2.5–4
Diameter of sori (mm)	0.5-0.9	0.6-0.9	0.5-0.8	0.7-1.0

Dodsworth 2000: fig. 103). The general form of the plants is comparable (Fig. 4) – both are medium-sized tree ferns with trunks reaching 8–10 m tall, covered in projecting stipe bases or stipe scars, and with fronds that are up to 4 m long and held horizontally (Oliver 1910: pl. XXI; Large & Braggins 2004: pls 41–44; Dawson & Lucas 2011: 112–113). The fronds are of very similar proportions and dissection (Table 2), and have stipe bases that are tuberculate and rough to the touch. Ecologically, *C. milnei* and *C. dealbata* are also similar, occurring as sub-canopy species in drier forest and open scrub.

The most obvious difference between the taxa (Table 4) is that in *Cyathea dealbata* the abaxial surface of the lamina is usually white, whereas the abaxial lamina surfaces in

C. milnei are green (Fig. 5). The scales and hairs on the abaxial surfaces of the costae are morphologically similar in both taxa, but proportionally there are fewer hairs and more scales in C. milnei than in C. dealbata (Fig. 5), and the scales of C. milnei are often bunched along the costae, obscuring the hairs. The sori of C. milnei are slightly larger than those of C. dealbata (0.7–1.0 mm cf. 0.5–0.8 mm in diameter) and the indusia are more fragile, less often forming a continuous rim. The dead fronds of C. milnei are more frequently persistent on the trunks than in C. dealbata, and the stipe bases are more conspicuously tuberculate (Fig. 6).

The most compelling difference between the taxa is the colour of the abaxial lamina surface, but even this is somewhat equivocal (Fig. 5). Young plants of *Cyathea* 



Fig. 1 Abaxial surfaces of lamina and costae of Cyathea cunninghamii (left) and C. kermadecensis (right).

Table 3 Distinguishing characters for Cyathea cunninghamii and C. kermadecensis.

	C. cunninghamii	C. kermadecensis
Colour of stipe base (Figs 2 and 3)	Black	Pale or red-brown
Tertiary pinnae (Fig. 1)	Divided up to 2/3 to midrib	Crenate
Indumentum on abaxial surface of costae (Fig. 1)	Red or colourless acaroid scales present	Red acaroid scales absent; colourless scales often present
	Ovate pale brown scales only scattered	Ovate pale brown scales abundant
	Pale brown ovate scales bearing red apical setae	Pale brown ovate scales lacking red apical setae
	Irregularly curled acicular hairs absent	Irregularly curled acicular hairs usually present



Fig. 2 The crown and trunk apices of Cyathea cunninghamii (left) and C. kermadecensis (right).



Fig. 3 Crown indumentum and stipe bases of Cyathea cunninghamii (left) and C. kermadecensis (right).



Fig. 4 Cyathea milnei (left) and C. dealbata (right) in cultivation at Otari-Wilton's Bush, Wellington.



Fig. 5 Colour of the abaxial surface of the lamina, and indumentum on the abaxial surfaces of the costae, on specimens of Cyathea dealbata from outside Northland (left) and within Northland (centre), and of C. milnei (right).

Table 4	Distinguishing	characters	for	Cyathea	dealbata and	C. milnei.

	C. dealbata	C. milnei
Dead fronds	Usually persistent only in young plants	Often persistent, forming a skirt around trunk
Colour of stipe base (Figs 6 and 7)	Usually whitish or pale brown	Pale brown or green
Surface of stipe base (Fig. 6)	Tuberculate, rough	Strongly tuberculate, very rough
Abaxial surface of lamina of mature plants (Fig. 5)	Usually white, rarely blue- or grey-green, or very rarely green	Green
Indumentum on abaxial	Curly hairs abundant	Curly hairs scattered
surface of secondary costae (Fig. 5)	Ovate pale brown scales occasional	Ovate pale brown scales abundant
Sori	Forming a deep cup at maturity, becoming shallow 0.5–0.8 mm diameter	Forming a deep cup at maturity, quickly breaking up 0.7–1.0 mm diameter

dealbata produce fronds with a green undersurface; white undersurfaces are produced only as the plants get older. Some populations of *C. dealbata* in northern New Zealand, from Raglan to North Cape, and on Coppermine Island and the Three Kings Islands, have mature laminae with blue-grey, grey-green or almost green abaxial surfaces. The coloration of the stipes varies similarly (Figs 6 and 7). The northern plants do not appear to differ in any other character from populations with white undersurfaces, except that plants with prostrate rhizomes have been reported from Warawara Forest (Rawlings 1969), from Warkworth (Bryony Macmillan, CHR 199046) and from Radar Bush (Peter de Lange, WELT P027464; de Lange 2004). Cyathea tricolor, described by Colenso (1883) from Seventy-mile Bush between Norsewood and Dannevirke but now reduced to synonymy with C. dealbata (Brownsey et al. 1985), was also noted for its 'bluish tint', as well as for its 'shining darkgreen upper foliage'. Occasional fertile fronds of C. dealbata that lack the white undersurface are also found.

The Australian species *Cyathea australis* (R.Br.) Domin is closely related to *C. dealbata* (Korall *et al.* 2007), and therefore also related to *C. milnei. Cyathea australis* differs morphologically most obviously from *C. dealbata* and *C. milnei* in lacking indusia, which are replaced by a fringe of scales around the sori (Bostock 1998: fig. 62D). It also

differs from *C. dealbata* by lacking the characteristic white underside to the laminae, although plants in Queensland sometimes have a glaucous surface (Bostock 1998). *Cyathea australis* is generally a much larger tree fern, with trunks to 20 m tall (Holttum 1964; Andrews 1990; Large & Braggins 2004: pls 22–23) and stipes to 800 mm long that have conical spines to 3 mm long (Bostock 1998). The hairs on the undersurfaces are much narrower and less abundant, and the scales rather smaller (generally less than 0.5 mm long) than in the New Zealand taxa. The morphological evidence therefore suggests that *C. milnei* and *C. dealbata* are more similar to each other than either is to *C. australis*.

### DNA sequences

There were no substitution differences in the *rbc*L or *trn*L-*trn*F sequences of *Cyathea kermadecensis* and *C. cunninghamii*. However, they did differ in the lengths of two mononucleotide runs, with *C. kermadecensis* having three fewer adenine bases at one mononucleotide run and one less adenine at a second mononucleotide run.

The only substitution differences amongst the *rbcL* and *trnL-trnF* sequences of *Cyathea milnei*, *C. australis* and *C. dealbata* were single (and separate) apomorphies for each of *C. australis* and *C. dealbata* in their *trnL-trnF* 



Fig. 6 The stipe bases of specimens of Cyathea dealbata from outside Northland (left) and within Northland (centre), and of C. milnei (right). Cyathea milnei is more tuberculate.



Fig. 7 The crown and trunk apices of specimens of Cyathea dealbata from outside Northland (left) and within Northland (centre), and of *C. milnei* (right).

sequences. There were also length differences at two mononucleotide runs, with *C. milnei* having one more adenine base than *C. dealbata* and one less than *C. australis* at one mononucleotide run, and three more thymine bases than *C. dealbata* and six more than *C. australis* at a second mononucleotide run.

### Discussion

Cyathea kermadecensis and C. milnei from the Kermadec Islands have long been recognised as separate species. On Raoul Island, they are easily distinguished from one another (Brownsey & Perrie 2015a): C. kermadecensis has hoodshaped mature indusia and lacks obvious curly hairs on the abaxial surface of the lamina, while C. milnei has cup-shaped mature indusia and abundant curly hairs on the abaxial surface of the lamina. Further differences include stipe colour (Figs 2 and 7), the form of the trunks (including the general persistence of dead fronds as a skirt on C. milnei, although this is not evident on the cultivated plant in Fig. 7) and ecology, as previously noted. However, the status of C. kermadecensis and C. milnei with respect to species from elsewhere has received little critical examination. Our study is the first comprehensive account of how they compare with the species to which they are each most closely related.

Substantial differences between the DNA sequences of the Kermadec Islands plants and the mainland plants could have been taken as support for their recognition as distinct species. However, in both pairs of species the DNA sequences are nearly invariable, and variation is of a level consistent with both infraspecific and interspecific differences that have been reported previously in ferns, particularly in the context of the deceleration of molecular evolution observed in tree ferns (Korall *et al.* 2010). These genetic data are therefore inconclusive as to whether the Kermadec Islands populations should be segregated as distinct species; however, they do reinforce the close relationships inferred from the morphology.

Morphological examination indicates that, in both cases, the Kermadec Islands populations can be distinguished consistently (Tables 3 and 4). The critical question then is whether the Kermadec Islands populations should be segregated taxonomically from their allopatric relatives, and, if so, at what rank – subspecies or species? In the case of *Cyathea cunninghamii* and *C. kermadecensis*, the former is a widespread species occurring in Australia from Tasmania to southern Queensland, and in New Zealand from

Fiordland to North Cape and on the Chatham Islands. *Cyathea cunninghamii*, or an ancestor, has evidently in the past also spread to the Kermadec Islands, where it has evolved in isolation some minor, but consistent, variation. The Kermadec Islands plants are morphologically more distinct from either the Australian or New Zealand plants than the latter two are from each other. With *C. dealbata* and *C. milnei*, it seems that morphological divergence has similarly occurred as a result of geographic isolation on Raoul Island.

In conclusion, the Kermadec Islands plants in both species pairs are morphologically distinct. However, we have no informative data as to their precise relationship to their mainland relatives, in particular as to whether they are reciprocally monophyletic or metaphyletic/paraphyletic (see Brownsey & Perrie 2014). Consequently, with no definitive evidence to the contrary, we adopt the taxonomically conservative approach of retaining both Cyathea kermadecensis and C. milnei as separate species in our treatment for the electronic Flora of New Zealand (Brownsey & Perrie 2015a). This includes full descriptions, based on specimens at AK, CHR and WELT (all annotated as seen for the Flora), and an identification key for all New Zealand Cyathea. We note, nevertheless, that future analyses that provide a more detailed understanding of the genealogy of the Raoul Island Cyathea may see their taxonomic rank revisited.

Cyathea kermadecensis and C. milnei are noteworthy in being among the c. 25 species of vascular plants endemic to the Kermadec Islands (Sykes 1977; West et al. 2010). The two Cyathea species nevertheless conform to a general pattern where most of the indigenous vascular plants of the subtropical Kermadec Islands are closely related to, if not conspecific with, those of temperate mainland New Zealand (Sykes & West 1996). This likely reflects the geological youthfulness (Pleistocene), instability (volcanism) and small size of the Kermadec Islands (Sykes 1977).

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