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PRIMER NOTE

SIMPLE SEQUENCE REPEAT MARKERS FOR KĀNUKA (*KUNZEA* SPP.; MYRTACEAE) PRESENT IN NEW ZEALAND[1]

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- *Premise of the study:* We developed simple sequence repeat (SSR) markers to facilitate population genetic studies on kānuka (*Kunzea* spp.; Myrtaceae).
- *Methods and Results:* A shotgun sequencing library was constructed from leaf material of *K. robusta* using a Roche 454 Junior sequencer, and a total of 3174 putative SSR regions were identified. Sixteen polymorphic markers were optimized for multiplex PCR on 10 endemic New Zealand *Kunzea* species. Each of these loci cross-amplified in all tested species. The amplified di-, tri-, and pentanucleotide repeats resulted in eight to 24 alleles per locus for a total of 220 specimens. The mean observed and expected heterozygosity per locus ranged from 0.18 to 0.77 and 0.33 to 0.82, respectively.
- *Conclusions:* The SSR markers we produced are valuable for phylogenetic and population studies on all endemic *Kunzea* spp. and may also be useful for studies on closely related *Kunzea* species from Australia.

Key words: kānuka; *Kunzea*; Myrtaceae; New Zealand; simple sequence repeat (SSR) markers.

The genus *Kunzea* Rchb. includes more than 60 shrub or small tree species from the Myrtaceae family endemic to New Zealand and Australia (WCSP, 2017). New Zealand *Kunzea* (kānuka) has recently been revised (de Lange, 2014), resulting in 10 *Kunzea* species endemic to New Zealand's islands: *K. amathicola* de Lange & Toelken, *K. ericoides* (A. Rich.) Joy Thomps., *K. robusta* de Lange & Toelken, and *K. serotina* de Lange & Toelken from both main islands; *K. linearis* (Kirk) de Lange & Toelken, *K. tenuicaulis* de Lange, and *K. toelkenii* de Lange from the North Island; *K. salterae* de Lange from Whale Island and Mayor Island; *K. sinclairii* (Kirk) W. Harris from Great Barrier Island; and *K. triregensis* de Lange from Three Kings Islands. Restricted geographic distribution and commercial use of these species (nectar for honey production and essential oils) have created a strong interest in their population genetics, but low genetic variation between these species makes phylogenetics difficult (de Lange, 2014). We used next-generation sequencing to develop novel simple sequence repeat markers (SSRs) for New Zealand *Kunzea* species. SSRs offer resolution of closely related species and populations while requiring short development time and low costs, and allow sample additions retrospectively. These markers will facilitate the generation of a national-scale population genetics data set to improve biodiversity and production management of kānuka.

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METHODS AND RESULTS

Molecular markers for *Kunzea* species were prepared following the method of Abdelkrim et al. (2009), with modifications. Total genomic DNA was extracted from 100 mg of fresh leaf material of *K. robusta* (CHR641860; Allan Herbarium [CHR], Lincoln, New Zealand) using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. With 410 ng of this DNA, a shotgun sequencing library was constructed for a Roche 454 Junior Genome Sequencer, a large-scale pyrosequencing system (Roche, Basel, Switzerland) at the Landcare Research Molecular Laboratory (Auckland, New Zealand). An average read length of 416 bp was obtained for 197,805 reads and a total yield of 82.3 Mb of sequence. We deposited the data in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI; accession no. SRR5342717). Di- to hexanucleotide repeat regions with at least four repeat units were identified with MSATCOMMANDER 0.8.2 (Faircloth, 2008). Primers were designed using Primer3 (Rozen and Skaletsky, 1999), implemented in MSATCOMMANDER, with the following specifications: 80–550 bp amplicon length, repeat units flanked by ≥50 bp, and 57–62°C melting temperature (Faircloth, 2008). From a total of 3174 putative simple sequence repeat regions, 96 primer pairs, providing a range of product sizes and repeat units, were screened. Adding an M13F tag (TGTAAAACGACGGCCAGT) to the 5′ end of the forward primers enabled the use of 6-FAM–labeled M13F probes in the second step of the PCR for economic genotyping (Schuelke, 2000; Abdelkrim et al., 2009).

All primer pairs were tested on *K. robusta* (sample used for library construction: CHR641860) and another four specimens: *K. robusta* (CHR688818), *K. serotina* (CHR641385), *K. ericoides* var. *linearis* (CHR553091), and *K. toelkenii* (CHR550085). DNA was extracted from 20 mg of dried leaf material using the NucleoSpin Plant II kit (PL1 lysis buffer; Macherey-Nagel, Düren, Germany) following manufacturer's instructions, resulting in 200–800 ng of DNA per sample. PCRs were performed in 15-µL reactions, containing 5–50 ng of DNA, and final concentrations of 0.08 µM forward primer, 0.32 µM reverse primer, 0.32 µM 6-FAM–labeled M13F primer, 1× KAPA plant PCR buffer with dNTPs, 0.3 units KAPA3G Plant DNA Polymerase (Kapa Biosystems, Wilmington, Massachusetts, USA), and PCR-grade H_2O. Thermocycling was conducted on

the Bioer GenePro thermocyler (Bioer Technology, Hangzhou, Zhejiang Province, China) using the following conditions: initial denaturation at 95°C for 5 min; 30 cycles of 95°C for 20 s, 55°C for 15 s, and 72°C for 30 s; followed by 10 cycles of 95°C for 20 s, 51°C for 15 s, and 72°C for 30 s; and final extension at 72°C for 10 min. Five-microliter PCR products were separated on 2.5% agarose gels. Concentration of PCR products was adjusted, and 1 µL added to 10 µL Hi-Di formamide (Applied Biosystems, Carlsbad, California, USA) and 0.2 µL GeneScan 600 LIZ Size Standard (Applied Biosystems). Samples were separated on a 3500xl genetic analyzer (Applied Biosystems) using a DS-33 dye set at the Landcare Research Molecular Laboratory. GeneMarker version 2.6.4 (SoftGenetics, State College, Pennsylvania, USA) was used for fragment sizing and scoring. After assessment of polymorphism and repeatability of each locus, 24 of the 96 loci tested produced diagnostic fragments with a maximum of two alleles per specimen.

PCRs were optimized for the integration of labeled forward primers (6-FAM, NED, VIC, or PET) to allow multiplex genotyping, and the M13F tail was omitted (Table 1). PCR reactions were set up as described above, omitting unlabeled forward primers. Thermocycling conditions were adjusted to: initial denaturation at 95°C for 5 min; followed by 35 cycles at 95°C for 20 s, 55°C for 15 s, and 72°C for 30 s; and a final extension at 72°C for 10 min. All 24 loci

TABLE 1. Characteristics of 24 polymorphic simple sequence repeat loci developed for New Zealand *Kunzea* species.

Locus[a]	Primer sequences (5′–3′)[b]	Repeat motif	Allele size range (bp)	Total *A* (*n* = 220)	Fluorescent dye[c,d]	Multiplex pool[c]	GenBank accession no.
Kanuka63	F: CACGTCGGAAAGTGATGGC	$(CTTTT)_4$	119–164	9	PET	1	KY352777
	R: GACAGCCAAACCCGCTTC						
Kanuka15	F: CTGCCGCTGCTAGGATACC	$(AAC)_9$	186–209	9	NED	1	KY352778
	R: GCAGGCATAGATTGAGGCG						
Kanuka29	F: GTCATGGTTATCCCTTCCATCG	$(AG)_{11}$	180–261	19	6-FAM	1	KY352779
	R: TTCGGTTTCCCGAACCCTC						
Kanuka38	F: TGCACTCCCTCACCTTGAC	$(AG)_{12}$	285–312	11	VIC	1	KY352780
	R: AACCACCAAACTCTTCGGC						
Kanuka67	F: AGCCTCAGTGACTAGCGATG	$(AGT)_8$	139–153	13	PET	2	KY352781
	R: AAGGTTCCTTCCCTTGGGC						
Kanuka94	F: CCGAGAATGGTTGCGTACC	$(AC)_{12}$	174–207	20	NED	2	KY352782
	R: CCTGCAGCCCTTAATCAGC						
Kanuka42	F: AAAGTTGGCAGTTGGGCAC	$(ACGGG)_4$	243–271	24	6-FAM	2	KY352783
	R: TCCGTCACTGTGGAAAGGG						
Kanuka71	F: GACTTTAAACAAGCACGTGGAC	$(AG)_{12}$	292–339	24	VIC	2	KY352784
	R: CCCTGGTCTTCCATTCAGTTTG						
Kanuka18	F: ACGAATGGGAAAGAGCCTAC	$(AG)_{10}$	182–220	19	PET	3	KY352785
	R: GCTGTCAGATAAATGGATTGGC						
Kanuka21	F: TTGTCCACTGCAAGGTTCC	$(GT)_{13}$	222–257	18	NED	3	KY352786
	R: TCTTTGGTCACATGCACTAGC						
Kanuka3	F: ACCAGAGCTCCGATTGCTC	$(AG)_{11}$	262–288	17	6-FAM	3	KY352787
	R: TCCGAAGCCCATCACTTCC						
Kanuka9	F: CTCACCTAACCAAGTGCTCG	$(AG)_{13}$	338–361	18	VIC	3	KY352788
	R: CCATCGTGGGCCTTTCTTG						
Kanuka11	F: GGAAGGTCACATGGTTGCC	$(AAACT)_4$	137–182	16	PET	4	KY352789
	R: CGATGCTGCGGGTTTATCG						
Kanuka4	F: AAGACATCGCTCGGGAAGC	$(CT)_{10}$	229–252	14	NED	4	KY352790
	R: TGCGGTTGTATTCTGTGCC						
Kanuka78	F: ACCTCTAAGGGACCCGAGG	$(AATTT)_4$	246–265	8	6-FAM	4	KY352791
	R: TCTCGTTGTTTGCGGATGAC						
Kanuka1	F: AGATTGCTCACTTGCCCAC	$(GT)_{11}$	310–326	20	VIC	4	KY352792
	R: ACCACCTGAGAATTGGAACC						
Kanuka7[e]	F: ACGGTCGTCGAATTCATGC	$(AAG)_8$	141–151	4	NA	NA	KY352793
	R: GCAACTGCTGCTTACCCTC						
Kanuka8[e]	F: TTCGTAAGCTCGGCGTTTG	$(CT)_{10}$	360–371	4	NA	NA	KY352794
	R: GGTGGAGTCAACGAGCAAG						
Kanuka52[e]	F: TCTTGAGAAATAACCCGATGTTC	$(GT)_{10}$	314–330	3	NA	NA	KY352795
	R: ACGTCAGACAAATCCTATCAACG						
Kanuka66[e]	F: TTAATTAGCCCAGCGATTTAGG	$(AG)_{10}$	200–210	4	NA	NA	KY352796
	R: TTGCAGATGGTTGCAAGTC						
Kanuka72[e]	F: AGGACCATAACAAGAACGATTGG	$(ATCCG)_4$	159–169	3	NA	NA	KY352797
	R: ACGGTGTGGACATGCAAAG						
Kanuka73[e]	F: GTGGATTCACCAAGACGGC	$(CTTTT)_4$	286–302	3	NA	NA	KY352798
	R: AGGAGCGTTGCATCAAAGG						
Kanuka74[e]	F: ACGTTGTTGCTTCGAACGG	$(ATC)_9$	260–282	3	NA	NA	KY352799
	R: CCACTCCCTCAGGACTACG						
Kanuka89[e]	F: ACGAAGTACAAATGCCACCG	$(ATT)_8$	219–249	3	NA	NA	KY352800
	R: GTGAAGAAGATCGAGCCAAGC						

Note: *A* = number of alleles.

[a] Annealing temperatures as per the Methods and Results section.

[b] M13F tag (TGTAAAACGACGGCCAGT) added to the 5′ end of each forward primer during initial screening.

[c] Initial amplification of test samples was carried out with 6-FAM–labeled M13F-tagged primers. As markers dropped out in multiplex PCR, a reference to fluorescent dye in multiplex and multiplex pool was not applicable; these markers are identified as "NA."

[d] Fluorescent dye used in multiplex.

[e] Data only from five initial test samples, as markers dropped out in multiplex PCR.

Table 2. Summary statistics for 16 polymorphic simple sequence repeat loci optimized for 10 New Zealand *Kunzea* species.[a]

Locus[b]	*K. amathicola* (n = 30; NI, SI)			*K. ericoides* (n = 30; NI, SI)			*K. tenuicaulis* (n = 25; NI)			*K. triregensis* (n = 1; Three Kings I)			*K. linearis* (n = 27; NI)			*K. robusta* (n = 32; NI, SI)			*K. salterae* (n = 10; Whale, Mayor I)			*K. serotina* (n = 28; NI, SI)			*K. sinclairii* (n = 22; Great Barrier I)			*K. toelkenii* (n = 15; NI)		
	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e
63	6	0.50	0.54	6	0.57	0.55	2	0.39	0.50	1	0.00	—	7	0.59	0.64	4	0.47	0.54	2	0.30	0.46	5	0.57	0.50	4	0.33	0.40	4	0.60	0.51
15	4	0.50	0.54	7	0.50	0.58	4	0.48	0.52	2	1.00	—	6	0.56	0.65	3	0.59	0.54	3	0.50	0.60	3	0.25	0.37	4	0.59	0.51	3	0.20	0.24
29	8	0.17	0.70	9	0.29	0.81	8	0.30	0.82	1	0.00	—	10	0.33	0.82	13	0.45	0.81	5	0.10	0.59	8	0.26	0.77	8	0.35	0.80	7	0.33	0.82
38	6	0.37	0.32	4	0.24	0.32	7	0.52	0.66	1	0.00	—	7	0.22	0.27	6	0.19	0.24	2	0.10	0.10	6	0.32	0.40	5	0.36	0.47	4	0.60	0.55
67	5	0.59	0.60	4	0.77	0.66	7	0.44	0.77	1	0.00	—	5	0.44	0.64	9	0.58	0.78	2	0.00	0.20	9	0.71	0.76	5	0.82	0.70	5	0.73	0.70
94	12	0.70	0.85	14	0.70	0.89	11	0.48	0.87	1	0.00	—	12	0.63	0.81	13	0.56	0.88	5	0.20	0.73	12	0.54	0.86	11	0.77	0.88	9	0.67	0.84
42	9	0.83	0.82	15	0.77	0.86	8	0.80	0.83	2	1.00	—	11	0.63	0.74	15	0.69	0.86	6	0.60	0.65	18	0.82	0.88	11	0.73	0.85	9	0.80	0.86
71	13	0.80	0.86	14	0.57	0.89	11	0.64	0.85	2	1.00	—	10	0.63	0.85	17	0.65	0.90	7	0.80	0.76	13	0.71	0.86	16	0.62	0.90	8	0.87	0.85
18	10	0.80	0.78	14	0.80	0.82	11	0.72	0.88	2	1.00	—	12	0.67	0.81	11	0.66	0.85	3	0.70	0.52	11	0.50	0.86	11	0.68	0.87	10	0.80	0.80
21	10	0.60	0.76	10	0.59	0.79	11	0.64	0.84	2	1.00	—	11	0.56	0.77	13	0.69	0.88	6	0.50	0.79	12	0.57	0.82	9	0.50	0.84	8	0.67	0.79
3	12	0.70	0.88	12	0.66	0.88	11	0.52	0.76	2	1.00	—	12	0.63	0.88	11	0.66	0.87	3	0.40	0.34	12	0.61	0.89	13	0.68	0.77	10	0.80	0.84
9	13	0.53	0.84	12	0.50	0.81	4	0.13	0.12	1	0.00	—	12	0.52	0.86	9	0.44	0.83	3	0.40	0.59	9	0.46	0.79	6	0.38	0.75	1	0.00	0.00
11	7	0.70	0.74	8	0.60	0.77	7	0.64	0.68	1	0.00	—	8	0.37	0.75	6	0.75	0.75	5	0.60	0.53	6	0.54	0.68	6	0.50	0.73	6	0.40	0.75
4	7	0.73	0.69	7	0.57	0.70	6	0.64	0.59	1	1.00	—	7	0.74	0.69	11	0.75	0.83	3	0.20	0.54	9	0.86	0.72	6	0.64	0.69	5	0.80	0.74
78	3	0.03	0.18	5	0.14	0.56	3	0.12	0.63	1	0.00	—	6	0.22	0.36	7	0.28	0.44	2	0.20	0.32	3	0.11	0.55	4	0.18	0.51	4	0.47	0.47
1	11	0.48	0.78	16	0.62	0.82	9	0.28	0.59	2	1.00	—	9	0.63	0.73	11	0.53	0.81	3	0.30	0.47	10	0.64	0.85	9	0.45	0.80	4	0.40	0.35

Note: A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; I = Island; n = number of individuals sampled; NI = North Island; SI = South Island.
[a] Locality and voucher information are provided in Appendix 1.
[b] Kānuka locus.

could be amplified, but only 16 were suitable for multiplex genotyping (Table 1), with the other loci showing a tendency to drop out in multiplex PCR. These markers may still have use for other populations or related taxa due to the observed polymorphism for the five test specimens producing three to four alleles per locus (Table 1). Leaf samples for up to 32 representatives for the 10 *Kunzea* species were sourced from the Auckland War Memorial Museum (AK) and CHR (Appendix 1). The collections provided only low numbers of specimens for rare populations of *K. toelkenii* (15), *K. salterae* (10), and *K. triregensis* (1). For the remaining kānuka species, specimens were selected from various populations representing a wide range of locations for each species in relation to the respective distribution across New Zealand. DNA was extracted using a JANUS work station (PerkinElmer, Waltham, Massachusetts, USA) following the manufacturer's instructions for the NucleoSpin Plant II kit (PL1 lysis buffer; Macherey-Nagel), resulting in 100–500 ng DNA per sample.

A total of 220 individuals of *Kunzea* species (Appendix 1) were successfully genotyped using the developed markers, with amplification products for at least 14 of 16 loci. Summary statistics were prepared in GenAlEx 6.501 (Peakall and Smouse, 2006) (Table 2). The developed kānuka SSR markers cross-amplified in all 10 *Kunzea* species and produced polymorphic bands in most species. Monomorphic bands were obtained for allele Kanuka9 for *K. toelkenii*. *Kunzea triregensis*, for which only one sample was available, resulted in two alleles for seven loci. The 10 to 32 individuals of the remaining nine *Kunzea* species produced eight to 24 alleles per locus (Table 1) and the mean observed and expected heterozygosity per locus across the species ranged from 0.18 (Kanuka78) to 0.77 (Kanuka42) and 0.33 (Kanuka38) to 0.82 (Kanuka71), respectively.

CONCLUSIONS

We developed 24 polymorphic SSR markers for New Zealand kānuka species, based on Roche 454 sequencing of total genomic DNA. We optimized 16 markers for multiplex genotyping of 10 *Kunzea* species endemic to New Zealand. The cross-species compatibility of these markers suggests suitability for other closely related species.

Despite low sample numbers per species and varying sample numbers per population, we observed high polymorphism in each species, indicating that the markers are valuable for intraspecific phylogenetic and population structure studies of kānuka.

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Appendix 1. Location data and herbarium voucher information for *Kunzea* species included in this study.

Species	Herbarium accession no.	New Zealand island	Latitude	Longitude	Coordinates estimated[a]
K. amathicola de Lange & Toelken	AK297617	NI	−36.5783333	174.3416667	N
K. amathicola	AK289686	SI	−40.5080556	172.7150000	N
K. amathicola	AK293310	NI	−34.8986111	173.0911111	N
K. amathicola	AK276552	NI	−36.5000000	174.6166667	N
K. amathicola	AK284417	NI	−37.8500000	174.7833333	N
K. amathicola	AK289231	NI	−40.6016667	175.2066667	N
K. amathicola	AK289243	SI	−40.5525000	173.0085850	Y
K. amathicola	AK297615	NI	−36.2744444	174.4355556	N
K. amathicola	AK289690	SI	−40.5525000	173.0085850	Y
K. amathicola	AK287967	NI	−35.1833333	173.1166667	N
K. amathicola	AK297613	NI	−36.4888889	174.5005556	N
K. amathicola	AK254924	NI	−38.0500000	174.8666667	N
K. amathicola	AK289328	NI	−40.6000000	175.1994444	N
K. amathicola	AK289687A	SI	−40.5208333	172.7419444	N
K. amathicola	AK282676	NI	−35.4368970	173.4820410	Y
K. amathicola	AK289241	SI	−40.6250000	172.6802778	N
K. amathicola	AK252352	NI	−36.3666667	174.0666667	N
K. amathicola	AK297614	NI	−36.5030556	174.6325000	N
K. amathicola	AK289331	NI	−38.0425000	174.7972222	N
K. amathicola	AK289679	NI	−40.6000000	175.1994444	N
K. amathicola	AK289235	SI	−40.7013889	172.3675000	N
K. amathicola	AK289683	SI	−40.5072222	172.6972222	N
K. amathicola	AK297616	NI	−36.3733333	174.3841667	N
K. amathicola	AK289242	SI	−40.6772222	172.6680556	N
K. amathicola	AK252276	NI	−36.3330000	174.1750000	Y
K. amathicola	AK297612	NI	−36.5002778	174.5119444	N
K. amathicola	AK298389	NI	−37.9770000	174.7890000	Y
K. amathicola	AK289230	NI	−40.6016667	175.2066667	N
K. amathicola	AK286080	SI	−40.5070000	172.6690000	Y
K. amathicola	AK289685	SI	−40.5044444	172.7113889	N
K. ericoides (A. Rich.) Joy Thomps. var. *linearis* (Kirk) W. Harris	AK228837	NI	−35.4833333	174.7333333	N
K. ericoides	AK286235	SI	−41.4166667	174.0166667	N
K. ericoides	AK358074	SI	−40.5077500	172.6602500	N
K. ericoides	AK202538	NI	−39.0166667	175.8000000	N
K. ericoides	CHR275438	NI	−38.9666667	176.2166667	Y
K. ericoides	CHR473166B	NI	−39.1750000	175.7633333	N
K. ericoides	CHR473168	NI	−39.3116667	175.7566667	N
K. ericoides	CHR61897	NI	−41.1666667	175.3166667	Y
K. ericoides	CHR592393	NI	−35.2666667	174.0666667	Y
K. ericoides	CHR416298	NI	−38.0166667	177.6166667	Y
K. ericoides	CHR473165	NI	−39.1566667	175.7700000	N
K. ericoides	CHR394491	SI	−40.8333333	172.6500000	Y
K. ericoides	CHR275542	SI	−40.6333333	172.5666667	Y
K. ericoides	CHR245659	NI	−37.1330000	175.5350000	Y
K. ericoides	CHR446816	NI	−36.7000000	174.6166667	Y
K. ericoides	CHR368871	NI	−39.5166667	174.4166667	Y
K. ericoides	CHR473162	NI	−40.9000000	176.0333333	N
K. ericoides	CHR468823	Great Barrier I	−36.1783333	175.4233333	N
K. ericoides	CHR67625	NI	−41.2500000	175.1166667	Y
K. ericoides	CHR244708	NI	−40.7000000	175.5833333	Y
K. ericoides	CHR471980	SI	−44.8200000	169.3250000	N
K. ericoides var. *linearis*	CHR468840B	NI	−34.9833333	173.1500000	Y
K. ericoides var. *linearis*	CHR468838	NI	−35.0600000	173.7480000	Y
K. ericoides	CHR201642	SI	−43.4166667	172.3166667	Y
K. ericoides	CHR471855	SI	−42.0700000	172.9316667	N
K. ericoides var. *linearis*[b]	CHR553091	NI	−36.3670000	174.1690000	Y
K. ericoides	AK289064	Three Kings I	−34.1644444	172.1308333	N
K. ericoides	AK289061	Three Kings I	−34.1619444	172.1375000	N
K. ericoides	AK289066	Three Kings I	−34.1644444	172.1308333	N
K. ericoides var. *linearis*	AK24092	Three Kings I	−34.1555550	172.1344780	Y
K. tenuicaulis de Lange	AK285267	NI	−36.8500000	174.7666667	N
K. tenuicaulis	AK285268	NI	−36.8500000	174.7666667	N
K. tenuicaulis	CHR550923A	NI	−38.0833333	176.7000000	N
K. tenuicaulis	CHR547023A	NI	−38.6500000	176.0666667	N
K. tenuicaulis	CHR276956	NI	−38.6166667	176.1000000	Y
K. tenuicaulis	CHR505949	NI	−38.4166667	176.1833333	N
K. tenuicaulis	CHR506319	NI	−38.6666667	176.0333333	N
K. tenuicaulis	CHR506236	NI	−38.4000000	176.2166667	N

Appendix 1. Continued.

Species	Herbarium accession no.	New Zealand island	Latitude	Longitude	Coordinates estimated[a]
K. tenuicaulis	CHR356386A	NI	−38.0500000	176.3500000	Y
K. tenuicaulis	CHR507223	NI	−38.3166667	176.3666667	N
K. tenuicaulis	CHR507220	NI	−38.3166667	176.3666667	N
K. tenuicaulis	AK288088	NI	−38.4000000	176.2166667	N
K. tenuicaulis	AK288101	NI	−38.6500000	176.0666667	N
K. tenuicaulis	AK286186	NI	−38.3166667	176.3833333	N
K. tenuicaulis	AK300912	NI	−37.8550000	176.9719444	N
K. tenuicaulis	AK288085	NI	−38.0833333	176.7000000	N
K. tenuicaulis	AK286152	NI	−38.6500000	176.0666667	N
K. tenuicaulis	AK288100	NI	−38.6500000	176.0666667	N
K. tenuicaulis	AK300909	NI	−37.8570000	176.9680000	Y
K. tenuicaulis	AK288083	NI	−38.6333333	176.1000000	N
K. tenuicaulis	AK288102	NI	−38.6500000	176.0666667	N
K. tenuicaulis	AK286156	NI	−38.1333333	176.2500000	N
K. tenuicaulis	AK288172	NI	−38.4000000	176.2166667	N
K. tenuicaulis	AK288099	NI	−38.4000000	176.2166667	N
K. tenuicaulis	AK253384	NI	−38.3666667	176.3666667	N
K. triregensis de Lange	AK226797	Three Kings I	−34.1530000	172.1330000	Y
K. linearis (Kirk) de Lange & Toelken	AK121371	NI	−34.4833333	172.8666667	N
K. linearis	AK287201	NI	−34.8750000	173.4010000	Y
K. linearis	AK287886	NI	−34.9000000	173.3500000	N
K. linearis	AK287881	NI	−34.9833333	173.3833333	N
K. linearis	AK288529	NI	−35.1833333	173.4500000	N
K. linearis	AK206328	NI	−35.2333333	173.4833333	N
K. linearis	AK287873	NI	−34.4000000	173.0166667	N
K. linearis	AK287877	NI	−34.4333333	172.6833333	N
K. linearis	AK176602	NI	−34.4166667	173.0166667	N
K. linearis	AK211064	NI	−34.8239480	173.1474740	Y
K. linearis	AK287853	NI	−34.8500000	173.4000000	Y
K. linearis	AK284582	NI	−34.9666667	173.3666667	N
K. linearis	AK287879	NI	−34.9940620	173.5289180	Y
K. linearis	AK287958	NI	−35.2166667	173.1333333	N
K. linearis	AK287947	NI	−36.8166667	174.7000000	N
K. linearis	AK288490	NI	−37.4500000	175.4666667	N
K. linearis	AK288490	NI	−37.4500000	175.4666667	N
K. linearis	AK288776	NI	−36.1988889	174.0594444	N
K. linearis	AK283237	NI	−36.1666667	174.6333333	N
K. linearis	AK286059	NI	−36.9000000	174.6500000	N
K. linearis	AK286054	NI	−37.3166667	175.4166667	N
K. linearis	AK283236	NI	−36.1666667	174.6333333	N
K. linearis	AK287025	NI	−36.4833333	174.6500000	N
K. linearis	AK297497	NI	−37.9930556	176.1741667	N
K. linearis	AK309446	NI	−36.3660000	174.1680000	Y
K. linearis	AK283245	NI	−36.1666667	174.6333333	N
K. linearis	AK254234	NI	−36.7820000	174.6520000	Y
K. robusta de Lange & Toelken[b,c]	CHR641860	SI	−43.6400306	172.4780500	Y
K. robusta	CHR551679A	NI	−39.1166667	177.0000000	N
K. robusta	CHR551738	NI	−37.6666667	177.8333333	N
K. robusta	CHR546981A	SI	−42.7333333	171.2000000	N
K. robusta	CHR551251	NI (Ponui I)	−36.8844444	175.1925000	N
K. robusta	CHR546982A	SI	−41.7500000	171.7166667	N
K. robusta	CHR546688A	NI	−39.3258333	174.1050000	N
K. robusta	CHR551683A	NI	−39.3666667	175.3333333	N
K. robusta	CHR550096	NI	−39.3833333	174.0500000	N
K. robusta	CHR546940A	NI	−38.9500000	177.3833333	N
K. robusta[b]	CHR688818	SI	−42.7666667	172.5500000	Y
K. robusta	AK289967	SI	−43.0166667	173.0833333	N
K. robusta	AK289984	SI	−45.8602778	170.5233333	N
K. robusta	AK283916	NI	−39.3166667	174.1000000	N
K. robusta	AK288048	NI	−39.9833333	176.0000000	N
K. robusta	AK297491	NI	−40.0761111	175.5988889	N
K. robusta	AK298622	NI	−40.6630556	176.2355556	N
K. robusta	AK298791	NI	−40.6280556	176.1641667	N
K. robusta	AK288592	SI	−41.3211111	174.1697222	N
K. robusta	AK288569	SI	−42.1666667	173.8833333	N
K. robusta	AK288444	SI	−42.4333333	171.3500000	N
K. robusta	AK286126	NI	−38.7833333	175.1333333	N
K. robusta	AK252130	SI	−43.7500000	172.8333333	N

Appendix 1. Continued.

Species	Herbarium accession no.	New Zealand island	Latitude	Longitude	Coordinates estimated[a]
K. robusta	AK289980	SI	–45.8600000	170.5219444	N
K. robusta	AK289154	NI	–39.2577778	173.9638889	N
K. robusta	AK288549	NI	–39.5000000	176.5000000	N
K. robusta	AK285568	SI	–45.8666667	170.5333333	N
K. robusta	AK285566	SI	–41.4166667	174.0166667	N
K. robusta 'East Cape'	AK299004	NI	–37.8141667	178.3797222	N
K. robusta 'East Cape'	AK298982	NI	–38.3822222	178.3322222	N
K. robusta 'East Cape'	AK288499	NI	–38.1666667	178.2666667	N
K. robusta 'East Cape'	AK269062	NI	–37.5833333	178.0833333	N
K. salterae de Lange	AK289814	NI (Whale I)	–37.8569444	176.9675000	N
K. salterae	AK283253	NI (Whale I)	–37.8500000	176.9666667	N
K. salterae	AK283250	NI (Whale I)	–37.8500000	176.9666667	N
K. salterae	AK284105	NI (Whale I)	–37.8500000	176.9666667	N
K. salterae	AK297561	NI (Whale I)	–37.8500000	176.9666667	N
K. salterae	AK289815	NI (Whale I)	–37.8525000	176.9683333	N
K. salterae	AK298088	NI (Whale I)	–37.8569444	176.9675000	N
K. salterae	AK289813	NI (Whale I)	–37.8552778	176.9675000	N
K. salterae	AK330883	NI (Mayor I)	–37.2869444	176.2713889	N
K. salterae	AK289816	NI (Whale I)	–37.8572222	176.9825000	N
K. serotina de Lange & Toelken[b]	CHR641385	SI	–42.7666667	172.5500000	Y
K. serotina	AK287554	SI	–42.1833333	172.2166667	N
K. serotina	AK288292	SI	–41.8500000	172.3333333	N
K. serotina	AK288543	SI	–42.8500000	172.6833333	N
K. serotina	AK288098	NI	–38.4833333	176.1333333	N
K. serotina	AK286264	NI	–38.7666667	176.2166667	N
K. serotina	AK288135	NI	–38.9333333	175.8666667	N
K. serotina	AK288239	NI	–39.4000000	176.3333333	N
K. serotina	AK286070	NI	–39.2500000	175.7666667	N
K. serotina	AK288134	NI	–38.9833333	175.7666667	N
K. serotina	AK288236	NI	–39.4000000	176.3166667	N
K. serotina	AK285572	NI	–39.1833333	175.7500000	N
K. serotina	AK288133	NI	–39.2833333	175.7333333	N
K. serotina	AK287551	SI	–41.8166667	172.4000000	N
K. serotina	CHR546949A	NI	–38.6166667	175.7166667	N
K. serotina	CHR551729	NI	–39.0500000	175.6000000	N
K. serotina	CHR546979A	NI	–38.9333333	175.8666667	N
K. serotina	CHR546945A	SI	–41.6333333	173.1166667	N
K. serotina	AK288547	NI	–38.6500000	176.0833333	N
K. serotina	AK138727	NI	–38.7500000	176.0833333	N
K. serotina	AK286262	SI	–41.6333333	173.0500000	N
K. serotina	AK285556	SI	–42.3500000	172.2333333	N
K. serotina	AK289970	SI	–42.3944444	172.4744444	N
K. serotina	AK348741	SI	–43.3525000	171.5558333	N
K. serotina	AK347652	NI	–38.2255556	176.5116667	N
K. serotina	AK288108	NI	–38.8833333	175.6000000	N
K. serotina	AK286136	SI	–42.5000000	172.8333333	N
K. serotina	AK286260	SI	–41.7166667	172.9000000	N
K. sinclairii (Kirk) W. Harris	AK242646	Great Barrier I	–36.2105556	175.3833333	N
K. sinclairii	AK278809	Great Barrier I	–36.1833333	175.4333333	N
K. sinclairii	AK242628	Great Barrier I	–36.1833333	175.3833333	N
K. sinclairii	AK288495	Great Barrier I	–36.1833333	175.4000000	N
K. sinclairii	AK289075	Great Barrier I	–36.2000000	175.4166667	N
K. sinclairii	AK245523	Great Barrier I	–36.2133333	175.3833333	N
K. sinclairii	AK287195	Great Barrier I	–36.1680000	175.4780000	Y
K. sinclairii	AK287857	Great Barrier I	–36.1833333	175.4166667	N
K. sinclairii	AK289074	Great Barrier I	–36.1952778	175.4180556	N
K. sinclairii	AK246813	Great Barrier I	–36.2105556	175.3833333	N
K. sinclairii	AK255943	Great Barrier I	–36.1833333	175.4833333	N
K. sinclairii	AK250789	Great Barrier I	–36.1833333	175.4166667	N
K. sinclairii	AK253369	Great Barrier I	–36.2000000	175.3833333	N
K. sinclairii	AK282635	Great Barrier I	–36.1833333	175.4166667	N
K. sinclairii	AK255946	Great Barrier I	–36.1833333	175.4833333	N
K. sinclairii	AK242634	Great Barrier I	–36.1833333	175.3833333	N
K. sinclairii	AK242667	Great Barrier I	–36.1833333	175.4833333	N
K. sinclairii	AK237883	Great Barrier I	–36.2166667	175.3833333	N
K. sinclairii	AK237880	Great Barrier I	–36.2166667	175.3833333	N
K. sinclairii	AK242652	Great Barrier I	–36.1833333	175.4833333	N
K. sinclairii	AK242674	Great Barrier I	–36.1833333	175.4833333	N
K. sinclairii	AK288322	Great Barrier I	–36.2166667	175.3833333	N
K. toelkenii de Lange	CHR550084	NI	–37.9000000	176.8333333	N

Appendix 1. Continued.

Species	Herbarium accession no.	New Zealand island	Latitude	Longitude	Coordinates estimated[a]
K. toelkenii[b]	CHR550085	NI	−37.9000000	176.8333333	N
K. toelkenii	AK300905	NI	−38.0090290	176.9919444	Y
K. toelkenii	AK287045	NI	−37.9000000	176.8333333	N
K. toelkenii	AK300904	NI	−38.0085026	177.1317053	Y
K. toelkenii	AK287049	NI	−37.9000000	176.8333333	N
K. toelkenii	AK300903	NI	−37.9411111	176.9883333	N
K. toelkenii	AK287047	NI	−37.9000000	176.8333333	N
K. toelkenii	AK301682	NI	−38.1133333	177.3791667	N
K. toelkenii	AK287048	NI	−37.9000000	176.8333333	N
K. toelkenii	AK299633	NI	−37.9150000	176.9025000	N
K. toelkenii	AK255350	NI	−37.9666667	176.8333333	N
K. toelkenii	AK299634	NI	−37.9180556	176.9219444	N
K. toelkenii	AK284553	NI	−37.9021130	176.8333333	Y
K. toelkenii	AK287042	NI	−37.9000000	176.8000000	N

Note: AK = Auckland War Memorial Museum; CHR = Allan Herbarium, Lincoln; I = Island; NI = North Island; SI = South Island.

[a] Collection records were checked carefully. When coordinates were not documented or did not match the location description, they were determined based on collector's notes.

[b] Used for initial primer screen.

[c] Used for library construction.