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

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The genus Kunzea Rchb. includes more than 60 shrub or small tree species from the Myrtaceae family endemic to New Zealand and Australia (WCSF, 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 Thompsons., 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. salteria 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.

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 Skaltsky, 1999), implemented in MSATCOMMANDER, with the following specifications: 80–550 bp amplicon length, repeat units flanked by 250 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 (TGTTAAAACGACGGCCAGT) 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 species: 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 (PLI 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 H2O. Thermocycling was conducted on

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the Bioer GenePro thermocycler (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 allowed, 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

| Locus | Primer sequences (5′–3′) | Repeat motif | Allele size range (bp) | Total A (n = 220) | Fluorescent dye<sup>ad</sup> | Multiplex pool<sup>bc</sup> | GenBank accession no. |
|-------|-------------------------|--------------|------------------------|--------------------|---------------------------|--------------------------|---------------------|
| Kanuka63 | F: CACGTCGGAAGATGATGAGCC<br>R: GACACGCAAAACCGCTTC | (CTTTT)<sub>4</sub> | 119–164 | 9 | PET | 1 | KY352777 |
| Kanuka15 | F: CTGGCCTGCTAAGTATAC<br>R: GACCGCATAGATTGGAG | (AAC)<sub>3</sub> | 186–209 | 9 | NED | 1 | KY352778 |
| Kanuka29 | F: GTAAGTTGTTGCCCTTCTCACAG<br>R: TGCCTGCTGCAATGGCTTC | (AG)<sub>11</sub> | 180–261 | 19 | 6-FAM | 1 | KY352779 |
| Kanuka38 | F: GAGACCTGCTGAGTGCTAC<br>R: AAGCCCAACCCCGCTTC | (AG)<sub>12</sub> | 285–312 | 11 | VIC | 1 | KY352780 |
| Kanuka67 | F: AGCTTCTGACTAGTAGT<br>R: AAGTTCTCTCCTTGAG | (AGT)<sub>3</sub> | 139–153 | 13 | PET | 2 | KY352781 |
| Kanuka94 | F: GCCAGAAGTGTGGGATCC<br>R: CACTCCCATTTACATTAGC | (ACGGG)<sub>4</sub> | 243–271 | 24 | 6-FAM | 2 | KY352783 |
| Kanuka71 | F: GACTTAAAAACACACCTTGAGC<br>R: CCTGTGCTTCTCTCTACATTT | (AG)<sub>12</sub> | 292–339 | 24 | VIC | 2 | KY352784 |
| Kanuka18 | F: AGCATGGGAAAGACGTCTAC<br>R: GCTGTGCAATAAAAGTGATG | (AG)<sub>10</sub> | 182–220 | 19 | PET | 3 | KY352786 |
| Kanuka21 | F: TTGTCCTGACATGCTAGCC<br>R: CCTTGGCTCACTGCATAGTG | (GT)<sub>13</sub> | 222–257 | 18 | NED | 1 | KY352786 |
| Kanuka3 | F: ACCAGAGTCCTGATGCTC<br>R: TCCGAACTCTGGAAGAGG | (AG)<sub>11</sub> | 262–288 | 17 | 6-FAM | 3 | KY352787 |
| Kanuka9 | F: CTCACAATACACTGATG<br>R: CCATGGGCGCTTTTCTTTT | (AG)<sub>11</sub> | 338–361 | 18 | VIC | 3 | KY352788 |
| Kanuka11 | F: GGAAGCTGCAATGTTGCTC<br>R: CAGGGTGGGCTTTGCTTAT | (AACT)<sub>4</sub> | 137–182 | 16 | PET | 4 | KY352789 |
| Kanuka4 | F: AGAGATCTGCAGTGCTGAGC<br>R: TGGCGGTTATCTATTTG | (CT)<sub>10</sub> | 229–252 | 14 | NED | 4 | KY352790 |
| Kanuka78 | F: ACTCCTAAAGGGACTCCGAG<br>R: TCTGTCTTGTGGGATGAC | (AAATT)<sub>4</sub> | 246–265 | 8 | 6-FAM | 4 | KY352791 |
| Kanuka1 | F: AGATTGCTCTTGCCCCC<br>R: ACCACTGAGAATTGAACC | (GT)<sub>11</sub> | 310–326 | 20 | VIC | 4 | KY352792 |
| Kanuka7<sup>e</sup> | F: AGGACTGGCAGATTTATG<br>R: GCACAGTCTGCTGAGG | (AAG)<sub>8</sub> | 141–151 | 4 | NA | NA | KY352793 |
| Kanuka8<sup>e</sup> | F: TTTGATGACAGTGCTGCTG<br>R: GGTGAAGTCAACACACTAC<br>R: GCAAGCCTGCTCTGATCC | (CT)<sub>10</sub> | 360–371 | 4 | NA | NA | KY352794 |
| Kanuka52 | F: TCTTGAGAAATACCCGATTTTC<br>R: AGCTGACCAAAATCTCAGAAC<br>R: TTAATTGAAGCTCCAGTTGAT<br>R: TTGCGAGATGTTGCAAGTC<br>R: AGGACCTAACAAGACGCTATGG<br>R: AGGCGTTGGCATCGAAG<br>R: AGGAGCGTTGCATCAAGAG<br>R: ACCCTCCATAAGACTGAC<br>R: AGCGATCTGCAAGC<br>R: GCTATGACAGTGCT<br>R: GCACAGTCTGCTGAGG | (ATCGG)<sub>4</sub> | 159–169 | 3 | NA | NA | KY352797 |
| Kanuka73 | F: GTGAGTTCCAAAGACG<br>R: AGGACTGGCAGATTTATG<br>R: GCACAGTCTGCTGAGG | (CTTTT)<sub>4</sub> | 286–302 | 3 | NA | NA | KY352798 |
| Kanuka74<sup>e</sup> | F: AGGACTGGCAGATTTATG<br>R: GCACAGTCTGCTGAGG | (ATC)<sub>3</sub> | 260–282 | 3 | NA | NA | KY352799 |
| Kanuka89<sup>e</sup> | F: AGCGATCTGCAAGC<br>R: GCTATGACAGTGCT<br>R: GCACAGTCTGCTGAGG | (ATT)<sub>3</sub> | 219–249 | 3 | NA | NA | KY352800 |

Note: A = number of alleles.
<sup>a</sup>Annealing temperatures as per the Methods and Results section.
<sup>b</sup>Initial amplification of test samples was carried out with 6-FAM–labeled M13F-tagged primers. As markers dropped out in multiplex PCR, a reference fluorescent dye in multiplex and multiplex pool was not applicable; these markers are identified as “NA.”
<sup>c</sup>Fluorescent dye used in multiplex.
<sup>d</sup>Data only from five initial test samples, as markers dropped out in multiplex PCR.

**Table 1.** Characteristics of 24 polymorphic simple sequence repeat loci developed for New Zealand *Kunzea* species.
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 in-species phylogenetic and population structure studies of kānuka.

### 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 in-specific phylogenetic and population structure studies of kānuka.

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**Table 2. Summary statistics for 16 polymorphic single-locus repeat loci optimized for 10 New Zealand *Kunzea* species.**

| Species          | n | A  | H_e | H_o | Locus  | Alleles | Heterozygosity |
|------------------|---|----|-----|-----|--------|---------|---------------|
| *K. salterae*    | 15| 6  | 0.50| 0.39| Kanuka 1 | 4 0.39 |              |
| *K. amathicola*  | 15| 6  | 0.50| 0.39| Kanuka 2 | 4 0.39 |              |
| *K. robusta*     | 15| 6  | 0.50| 0.39| Kanuka 3 | 4 0.39 |              |
| *K. triregens*   | 15| 6  | 0.50| 0.39| Kanuka 4 | 4 0.39 |              |
| *K. salterae*    | 15| 6  | 0.50| 0.39| Kanuka 5 | 4 0.39 |              |
| *K. amathicola*  | 15| 6  | 0.50| 0.39| Kanuka 6 | 4 0.39 |              |
| *K. robusta*     | 15| 6  | 0.50| 0.39| Kanuka 7 | 4 0.39 |              |
| *K. triregens*   | 15| 6  | 0.50| 0.39| Kanuka 8 | 4 0.39 |              |
| *K. salterae*    | 15| 6  | 0.50| 0.39| Kanuka 9 | 4 0.39 |              |
| *K. amathicola*  | 15| 6  | 0.50| 0.39| Kanuka10 | 4 0.39 |              |
| *K. robusta*     | 15| 6  | 0.50| 0.39| Kanuka11 | 4 0.39 |              |
| *K. triregens*   | 15| 6  | 0.50| 0.39| Kanuka12 | 4 0.39 |              |
| *K. salterae*    | 15| 6  | 0.50| 0.39| Kanuka13 | 4 0.39 |              |
| *K. amathicola*  | 15| 6  | 0.50| 0.39| Kanuka14 | 4 0.39 |              |
| *K. robusta*     | 15| 6  | 0.50| 0.39| Kanuka15 | 4 0.39 |              |
| *K. triregens*   | 15| 6  | 0.50| 0.39| Kanuka16 | 4 0.39 |              |

Note: A: number of alleles; H_e: expected heterozygosity; H_o: observed heterozygosity.
### 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* |
|---------|------------------------|--------------------|----------|-----------|------------------------|
| *K. amathicola* de Lange & Toelken | AK297617 | NI | −36.5783333 | 174.3416667 | N |
| *K. amathicola* | AK289868 | SI | −40.5080556 | 172.7150000 | N |
| *K. amathicola* | AK293310 | NI | −34.8961111 | 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 | −40.5520000 | 173.0085850 | Y |
| *K. amathicola* | AK297615 | NI | −36.2744444 | 174.4355556 | N |
| *K. amathicola* | AK289690 | SI | −40.5520000 | 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* | AK289678A | SI | −40.5208333 | 172.7419444 | Y |
| *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* | CHR275538 | NI | −38.9666667 | 176.2166667 | N |
| *K. ericoides* | CHR473165 | NI | −40.9000000 | 176.0333333 | N |
| *K. ericoides* | CHR473162 | NI | −40.5000000 | 175.2066667 | N |
| *K. ericoides* | CHR473167 | NI | −39.1566667 | 175.7700000 | N |
| *K. ericoides* | CHR67625 | NI | −41.2500000 | 174.9166667 | N |
| *K. ericoides* | CHR471604 | SI | −40.7000000 | 175.5833333 | Y |
| *K. ericoides* | CHR471605 | SI | −40.7000000 | 175.5833333 | Y |
| *K. ericoides* var. *linearis* | CHR468823 | Great Barrier I | −36.1783333 | 175.2333333 | N |
| *K. ericoides* | CHR67625 | NI | −41.2500000 | 175.1166667 | Y |
| *K. ericoides* var. *linearis* | CHR244708 | NI | −40.7000000 | 175.5833333 | Y |
| *K. ericoides* | CHR471604 | SI | −40.7000000 | 175.5833333 | Y |
| *K. ericoides* | CHR471605 | SI | −40.7000000 | 175.5833333 | Y |
| *K. ericoides* | CHR468823 | Great Barrier I | −36.1783333 | 175.2333333 | N |
| *K. ericoides* var. *linearis* | CHR530911 | NI | −36.3670000 | 174.1690000 | Y |
| *K. ericoides* | AK289064 | Three Kings I | −34.1644444 | 172.1308333 | N |
| *K. ericoides* | AK289061 | Three Kings I | −34.1644444 | 172.1308333 | N |
| *K. ericoides* var. *linearis* | AK24092 | Three Kings I | −34.1555550 | 172.1344790 | N |
| *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 | −38.0833333 | 176.7000000 | N |
| *K. tenuicaulis* | CHR76956 | NI | −38.6166667 | 176.1000000 | Y |
| *K. tenuicaulis* | CHR50949 | 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 estimateda |
|---------------|-------------------------|--------------------|----------|-----------|------------------------|
| *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 | −38.4000000 | 176.2166667 | N                      |
| *K. tenuicaulis* | AK300909     | NI | −38.4000000 | 176.2166667 | N                      |
| *K. tenuicaulis* | AK288085     | NI | −38.8033333 | 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* | AK253384     | NI | −38.3666667 | 176.3666667 | N                      |
| *K. tenuicaulis* | AK226797     | Three Kings I | −34.1530000 | 172.1330000 | Y                      |
| *K. linearis*    | AK121371     | NI | −34.4833333 | 172.8666667 | N                      |
| *K. linearis*    | AK287853     | NI | −34.8500000 | 173.4000000 | Y                      |
| *K. linearis*    | AK287879     | NI | −34.9940620 | 173.5289180 | Y                      |
| *K. linearis*    | AK287877     | NI | −35.1833333 | 172.6833333 | N                      |
| *K. linearis*    | AK288776     | NI | −35.2333333 | 173.4833333 | N                      |
| *K. linearis*    | AK287737     | NI | −35.4333333 | 172.8333333 | N                      |
| *K. linearis*    | AK287789     | NI | −36.0000000 | 174.0666667 | N                      |
| *K. linearis*    | AK288490     | NI | −37.4500000 | 175.4666667 | Y                      |
| *K. linearis*    | AK288491     | NI | −37.4500000 | 175.4666667 | N                      |
| *K. linearis*    | AK288776     | NI | −39.2666667 | 174.6333333 | N                      |
| *K. linearis*    | AK283054     | NI | −36.9000000 | 174.0500000 | N                      |
| *K. linearis*    | AK283054     | NI | −37.3166667 | 175.4166667 | N                      |
| *K. linearis*    | AK287326     | NI | −36.6666667 | 174.6333333 | N                      |
| *K. linearis*    | AK287025     | NI | −36.4833333 | 174.6500000 | N                      |
| *K. linearis*    | AK297497     | NI | −37.9930556 | 174.1466667 | N                      |
| *K. linearis*    | AK309446     | NI | −36.3600000 | 174.1680000 | Y                      |
| *K. linearis*    | AK283245     | NI | −36.1666667 | 174.6333333 | N                      |
| *K. linearis*    | AK254234     | NI | −36.7800000 | 174.6200000 | Y                      |
| *K. robusta de Lange & Toelken* | CHR61860 | SI | −43.6403060 | 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) | −38.8444444 | 175.1925000 | N                      |
| *K. robusta*     | CHR546982A   | SI | −41.7500000 | 171.7166667 | N                      |
| *K. robusta*     | CHR546688A   | SI | −39.3258333 | 174.1000000 | N                      |
| *K. robusta*     | CHR551683A   | SI | −39.3666667 | 175.3333333 | N                      |
| *K. robusta*     | CHR550096    | SI | −39.3833333 | 174.0500000 | N                      |
| *K. robusta*     | CHR546940A   | NI | −39.8500000 | 174.3833333 | N                      |
| *K. robusta*     | 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 | −40.0711111 | 175.5988889 | N                      |
| *K. robusta*     | AK298622     | NI | −40.6305556 | 176.2555556 | N                      |
| *K. robusta*     | AK298791     | NI | −40.6205556 | 176.1616667 | N                      |
| *K. robusta*     | AK288592     | SI | −41.3211111 | 174.2111111 | N                      |
| *K. robusta*     | AK288657     | SI | −42.1666667 | 173.8833333 | N                      |
| *K. robusta*     | AK288444     | SI | −42.4333333 | 171.3500000 | N                      |
| *K. robusta*     | AK286126     | SI | −38.7833333 | 175.1333333 | N                      |
| *K. robusta*     | AK252130     | SI | −43.7500000 | 172.8333333 | N                      |
| Species | Herbarium accession no. | New Zealand island | Latitude | Longitude | Coordinates estimated |
|---------|------------------------|--------------------|----------|-----------|-----------------------|
| *K. robusta* | AK289980 | SI | −45.8600000 | 170.5219444 | Y                     |
| *K. robusta* | AK289154 | NI | −39.2577778 | 173.9638889 | Y                     |
| *K. robusta* | AK288549 | NI | −39.5000000 | 176.5000000 | Y                     |
| *K. robusta* | AK285568 | SI | −45.8666667 | 170.5333333 | Y                     |
| *K. robusta* | AK285566 | SI | −41.4166667 | 174.0166667 | Y                     |
| *K. robusta* 'East Cape' | AK299004 | NI | −37.8141667 | 178.3797222 | Y                     |
| *K. robusta* 'East Cape' | AK298982 | NI | −38.3822222 | 178.3222222 | Y                     |
| *K. robusta* 'East Cape' | AK288499 | NI | −38.1666667 | 178.0000000 | Y                     |
| *K. robusta* 'East Cape' | AK269062 | NI | −37.5833333 | 178.0833333 | Y                     |
| *K. salterae de Lange* | AK289814 | NI (Whale I) | −37.8569444 | 176.9675000 | Y                     |
| *K. salterae* | AK283253 | NI (Whale I) | −37.8500000 | 176.9666667 | Y                     |
| *K. salterae* | AK283250 | NI (Whale I) | −37.8500000 | 176.9666667 | Y                     |
| *K. salterae* | AK284105 | NI (Whale I) | −37.8500000 | 176.9666667 | Y                     |
| *K. salterae* | AK297561 | NI (Whale I) | −37.8500000 | 176.9666667 | Y                     |
| *K. salterae* | AK298088 | NI (Whale I) | −37.8569444 | 176.9675000 | Y                     |
| *K. salterae* | AK289815 | NI (Whale I) | −37.8525000 | 176.9683333 | Y                     |
| *K. salterae* | AK289813 | NI (Whale I) | −37.8552778 | 176.9675000 | Y                     |
| *K. salterae* | AK330883 | NI (Mayor I) | −37.2869444 | 176.2713889 | Y                     |
| *K. serotina de Lange & Toelken* | CHR641385 | SI | −42.7666667 | 172.5500000 | Y                     |
| *K. serotina* | AK287554 | SI | −42.1833333 | 172.2166667 | Y                     |
| *K. serotina* | AK288292 | SI | −42.8500000 | 172.6833333 | Y                     |
| *K. serotina* | AK288543 | SI | −39.4000000 | 176.7166667 | Y                     |
| *K. serotina* | AK286264 | NI | −38.7666667 | 176.2166667 | Y                     |
| *K. serotina* | AK288135 | NI | −38.9333333 | 175.8666667 | Y                     |
| *K. serotina* | AK288239 | NI | −39.4000000 | 176.7166667 | Y                     |
| *K. serotina* | AK286070 | NI | −39.2500000 | 175.7666667 | Y                     |
| *K. serotina* | AK288134 | NI | −38.9833333 | 175.7666667 | Y                     |
| *K. serotina* | AK288236 | NI | −39.4000000 | 176.3166667 | Y                     |
| *K. serotina* | AK285572 | NI | −39.1833333 | 175.7333333 | Y                     |
| *K. serotina* | AK288133 | NI | −39.2833333 | 175.7666667 | Y                     |
| *K. serotina* | AK288239 | NI | −39.4000000 | 176.7166667 | Y                     |
| *K. serotina* | AK285566 | SI | −41.6333333 | 173.0500000 | Y                     |
| *K. serotina* | AK285556 | SI | −42.3944444 | 172.4744444 | Y                     |
| *K. serotina* | AK348741 | SI | −43.3525000 | 171.5558333 | Y                     |
| *K. serotina* | AK347652 | NI | −38.6500000 | 176.0833333 | Y                     |
| *K. serotina* | AK288108 | NI | −38.8833333 | 175.6000000 | Y                     |
| *K. serotina* | AK286136 | SI | −42.5000000 | 172.2166667 | Y                     |
| *K. serotina* | AK286260 | SI | −41.7166667 | 172.9000000 | Y                     |

Appendix 1. Continued.
### Appendix 1. Continued.

| Species         | Herbarium accession no. | New Zealand island | Latitude   | Longitude   | Coordinates estimated¹ |
|-----------------|--------------------------|--------------------|------------|-------------|------------------------|
| *K. toelkenii⁵* | CHR500085                | NI                 | −37.900000 | 176.833333  | N                      |
| *K. toelkenii*  | AK300905                 | NI                 | −38.009029 | 176.919444  | Y                      |
| *K. toelkenii*  | AK287045                 | NI                 | −37.900000 | 176.833333  | N                      |
| *K. toelkenii*  | AK300904                 | NI                 | −38.008526 | 177.131705  | Y                      |
| *K. toelkenii*  | AK287049                 | NI                 | −37.900000 | 176.833333  | N                      |
| *K. toelkenii*  | AK300903                 | NI                 | −37.941111 | 176.988333  | N                      |
| *K. toelkenii*  | AK287047                 | NI                 | −37.900000 | 176.833333  | N                      |
| *K. toelkenii*  | AK301682                 | NI                 | −38.113333 | 177.379167  | N                      |
| *K. toelkenii*  | AK287048                 | NI                 | −37.900000 | 176.833333  | N                      |
| *K. toelkenii*  | AK299633                 | NI                 | −37.915000 | 176.902500  | N                      |
| *K. toelkenii*  | AK255350                 | NI                 | −37.966667 | 176.833333  | N                      |
| *K. toelkenii*  | AK299634                 | NI                 | −37.918056 | 176.921944  | N                      |
| *K. toelkenii*  | AK284553                 | NI                 | −37.902130 | 176.833333  | Y                      |
| *K. toelkenii*  | AK287042                 | NI                 | −37.900000 | 176.800000  | N                      |

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

¹Collection records were checked carefully. When coordinates were not documented or did not match the location description, they were determined based on collector’s notes.

²Used for initial primer screen.

³Used for library construction.