Three species of Selliera Cav. (Goodeniaceae), a small genus of rhizomatous perennial herbs, are currently recognized. The most common, S. radicans Cav., was originally described from Australia and also occurs in Chile and New Zealand. Within New Zealand, the species is relatively common along much of the coast and occurs less frequently in inland freshwater habitats. Selliera radicans was described by Allan (1961) as “polymorphic, with a considerable range of leaf form and size.” Ensuing investigation (Ogden, 1974) into the polymorphic nature of S. radicans distinguished an estuarine form and a dune form based on differences in rhizome formation, growth form, and leaf shape, the last of which is the most conspicuous. The estuarine form is typical of S. radicans, having elongated spatulate leaves, whereas the dune form has shorter rotund leaves. Ogden (1974) performed common garden studies and determined that the leaf form difference is strongly genetically determined and, on this basis, suggested that the two forms be regarded as distinct ecotypes. Based on further taxonomic investigations, Heenan (1997) later raised the dune ecotype to a distinct species, S. rotundifolia Heenan; however, obvious hybrid swarms between S. radicans and S. rotundifolia have been observed at sites of sympatry.

The third species in the genus, S. microphylla Colenso, was described from two regions of New Zealand in 1890 (Colenso, 1890). This species is morphologically similar to but distinguished from S. radicans primarily by a smaller form; however, this difference appears to be a plastic developmental response to the environment, as it disappears when field-collected individuals are grown in a common greenhouse (Symonds and Pilkington, pers. obs.). Based on a single individual, S. microphylla has a distinct chromosome number (2n = 56; Murray and de Lange, 2013) relative to S. radicans and S. rotundifolia (both 2n = 16; Dawson, 2000).

Given various degrees of morphological overlap, developmental plasticity, sympatry, and hybridization, genetic distinction among these species and, therefore, taxonomic status warrant further investigation. Here, using 454 pyrosequencing, microsatellite markers were developed for Selliera species for use in assessing genetic structure within and investigating hybridization among Selliera species in future work.

**METHODS AND RESULTS**

DNA from S. radicans collected from a population at Moana Roa beach (Appendix 1) was chosen for 454 sequencing. Genomic DNA was extracted from silica gel–dried leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) method with an initial sucrose-Tris-EDTA (STE) wash (Shepherd and McLay, 2011) and an additional RNase step. The resulting DNA was dissolved in 100 μL of TE buffer. The sample had a concentration of 84.8 ng/μL and a 260/280 absorbance reading of 1.99 as measured on a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The DNA was run on a 1% agarose gel to assess DNA quality and ensure that RNA had been removed successfully. Approximately 5 μg of this DNA was used to construct a shotgun genomic DNA library that was sequenced in a full run on a 454 GS FLX system (454 Life Sciences, a Roche Company, Branford, Connecticut, USA) by New Zealand Genomics Ltd.

The 454 sequencing run generated more than 23 Mbp of quality data, with 57,561 sequences averaging 407 bp in length. The sequence data were assembled into contigs in Geneious (version 5.6.7; Kearse et al., 2012) to increase the efficiency of microsatellite detection and to prevent locus duplication. The assembly yielded 8101 contigs with an average sequence length of 672 bp. MSATCOM- MANDER version 0.8.2 (Faircloth, 2008) was used to search the Selliera contigs for di-, tri-, and tetranucleotide repeat motifs with a minimum of seven uninterrupted repeats and with the requirement to design primers at least 50 bp from the repeat region using Primer3 (Rozen and Skaletsky, 1999). Criteria for primer pair
From the 107 primer pairs, 43 were selected for initial testing based on a refinement of criteria, including a maximum number of uninterrupted repeats (12), primer melting temperatures, and overall maximum repeat length. Selected primer pairs were manufactured by IDT (Coralville, Iowa, USA) and screened initially on 15 individuals representing multiple populations of *S. radicans*, *S. rotundifolia*, and *S. microphylla*. PCR amplification was performed in a volume of 10 μL with 1× buffer BD (Solis BioDyne, Tartu, Estonia), 50 μM of each dNTP, 2.5 μM MgCl₂, 0.5 units of FIREPol DNA polymerase (Solis BioDyne), 20 nM of forward primer, 450 nM of reverse primer, and 450 nM M13 tail primer labeled with FAM (see Schuelke, 2000 for M13-tailed PCR). Amplification by PCR was attained by: 95°C for 3 min; 35 cycles of 95°C for 30 s, 53°C for 30 s, 72°C for 30 s. PCR amplification was performed in a volume of 10 μL with 1× buffer BD (Solis BioDyne, Tartu, Estonia), 50 μM of each dNTP, 2.5 μM MgCl₂, 0.5 units of FIREPol DNA polymerase (Solis BioDyne), 20 nM of forward primer, 450 nM of reverse primer, and 450 nM M13 tail primer labeled with FAM (see Schuelke, 2000 for M13-tailed PCR). Amplification by PCR was attained by: 95°C for 3 min; 35 cycles of 95°C for 30 s, 53°C for 30 s.

### Table 1. Characteristics of 15 microsatellite marker primer pairs developed from *Selliera radicans*.

| Locus | Primer sequences (5’–3’) | Repeat motif | Allele size range (bp) | Pooling group/dye | GenBank accession no. |
|-------|--------------------------|--------------|------------------------|-------------------|----------------------|
| SR3b  | TGCTGTTAGCTCCCTGGGAG     | (TC)₇        | 196                    | NA                | KU589266             |
| SR4b  | CCCTCTCCTGACCTTCCA       | (TA)₇        | 302                    | NA                | KU589267             |
| SR6   | GAGCTGGCTTCCCTGGC        | (GA)₈        | 208–214                | 2/NED             | KU522441             |
| SR11c,d | GCTCCCTGATCCCTGATGAGG   | (GT)₈        | 182–194                | 2/VIC             | KU522442             |
| SR13c,d | AGGCTCTGTTAGCTGCTGAGAC  | (TC)₉        | 313–315                | 4/FAM             | KU522443             |
| SR17  | AATGAAAAGACCAATCCCCAAA   | (AT)₁₂       | 252–258                | 1/FAM             | KU522444             |
| SR24  | GGAAGTGAATATGGGGCA       | (AT)₈        | 203–209                | 3/NED             | KU522445             |
| SR29c,d | GAAGCGCCGACAACTACCC     | (AG)₇        | 397–399                | 3/VIC             | KU522446             |
| SR31  | CGAGGGGATGGTATCCCTCC     | (CT)₁₁       | 369–373                | 1/NED             | KU522447             |
| SR33b  | TAAGGAGGGCCGCTTTCG       | (AT)₉        | 235                    | NA                | KU589268             |
| SR35b  | TGGTGCGACTATGCGACG       | (AT)₉        | 177                    | NA                | KU589269             |
| SR37c  | TCCAGCTTTGGCGAAGAAGTGGTC | (AG)₁₀       | 296–302                | 2/FAM             | KU522448             |
| SR41b  | CAGTTCCGCCAGAACATCTGAC   | (AC)₁₂       | 352                    | NA                | KU589270             |
| SR45  | GTGGTGATCCGCTATCCAGG     | (GAT)₁₀      | 271–283                | 1/FAM             | KU522449             |
| SR46d  | AAGAACACGCCGACCAAAAAGC   | (TA)₁₁       | 253–267                | 4/VIC             | KU522450             |

**Note:** NA = not applicable. 
* Monomorphic locus in *S. radicans*. 
* Monomorphic locus in *S. rotundifolia*. 
* Monomorphic locus in *S. microphylla*. 
* Marker pooling and dye combinations.

### Table 2. Results of primer screening in populations of *Selliera radicans*.a

| Locus | A | H₀ | Hₑ | A | H₀ | Hₑ | A | H₀ | Hₑ | Total | H₀ | Hₑ |
|-------|---|----|----|---|----|----|---|----|----|-------|---|----|
| SR6   | 1 | 0  | 0  | 0 | 1  | 0  | 0 | 2  | 1.00| 0.50 | 3  | 0.15| 0.26|
| SR11  | 1 | 0  | 0  | 0 | 1  | 0  | 0 | 2  | 1.00| 0.50 | 3  | 0.48| 0.39|
| SR13  | 1 | 0  | 0  | 0 | 1  | 0  | 0 | 2  | 1.00| 0.50 | 3  | 0.23|
| SR17  | 1 | 0  | 0  | 0 | 1  | 0  | 0 | 2  | 0.99| 0.19 | 1  | 0   | 0.55|
| SR24  | 2 | 1.00| 0.50| 2 | 0.42| 0.39| 2 | 0  | 0.50| 1  | 0   | 4   | 0.37| 0.66|
| SR29  | 1 | 0  | 0  | 0 | 1  | 0  | 0 | 1  | 0.00| 0  | 2   | 0   | 0.23|
| SR31  | 1 | 0  | 0  | 0 | 1  | 0  | 0 | 1  | 0.00| 0  | 2   | 0   | 0.17|
| SR37  | 1 | 0  | 0  | 0 | 2  | 0.70| 0.50| 4 | 0.42| 0.63| 2  | 1.00| 0.50| 7  | 0.46| 0.80|
| SR45  | 2 | 0.05| 0.14| 2 | 0.10| 0.10| 3 | 0.74| 0.65| 1  | 0   | 0   | 4  | 0.25| 0.68|
| SR46  | 2 | 0.05| 0.05| 5 | 0.79| 0.63| 3 | 0.79| 0.66| 2  | 1.00| 0.50| 7  | 0.59| 0.76|

**Note:** A = number of alleles; H₀ = expected heterozygosity; Hₑ = observed heterozygosity.

aVoucher information is provided in Appendix 1.
markers are polymorphic within *S. radicans* and also amplify from the New Zealand congeners *S. rotundifolia* and *S. microphylla*. These 10 markers will be used in future studies of population structure and hybridization in the genus.

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**APPENDIX 1.** Voucher information for *Selliera* populations analyzed in this study.

| Species          | Population locality | GPS coordinates       | N  | Plant ID/Voucher |
|------------------|---------------------|-----------------------|----|-----------------|
| *Selliera radicans* | Napier              | −39°28′4.15″S, 176°52′30.14″E | 20 | MPN 47843       |
| *Selliera radicans* | Ohwiwa               | −37°59′14.38″S, 177°9′37.47″E | 20 | MPN 47844       |
| *Selliera radicans* | Tauranga             | −37°43′27.84″S, 176°11′25.04″E | 20 | MPN 47840       |
| *Selliera radicans* | Australia            | −37°49′37.99″S, 144°59′2.00″E | 10 | MPN 48358       |
| *Selliera rotundifolia* | Himatangi         | −40°21′44.53″S, 175°13′57.32″E | 20 | MPN 47848       |
| *Selliera microphylla* | Central volcanic plateau (CVP) | −38°54′34.95″S, 176°27′20.8″E | 20 | MPN 47841       |

*Vouchers deposited at the Dame Ella Campbell Herbarium (MPN), Massey University, Palmerston North, New Zealand.*