# A population genetics approach to species delimitation in the genus *Selliera* (Goodeniaceae).

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## ABSTRACT

*Selliera* is a genus in the Goodeniaceae described as a small creeping herb. Currently there is only one internationally recognised species of *Selliera, Selliera radicans*. In New Zealand, three species have been described based on morphology and geographic location although there is disagreement about whether these actually constitute different species. *Selliera rotundifolia* is distinguished from *S. radicans* by rounder leaves and a preferred dune habitat compared to the estuary habitat of *S. radicans. Selliera microphylla* is distinguished from *S. radicans* by a smaller size and inland location. However, *S. microphylla* reverts to a size similar to *S. radicans* when grown in the same environment, but a single chromosome count for *S. microphylla* on the Central Volcanic Plateau is 2*n*=56. Both *S. rotundifolia* and *S. radicans* have chromosome counts of 2*n*=16. Species delimitation is important in biology, conservation, and evolutionary studies but remains a difficult task. I applied a population genetics approach combined with morphological analysis of leaves and existing karyotype data to determine the species boundaries within *Selliera.*

Microsatellite markers are ideal for use in population genetics due to the higher mutation rate, genotyping ease and their co-dominant nature. No microsatellite markers previously existed for use in *Selliera.* In this study, next generation sequencing was used to develop microsatellite markers for *Selliera.* From 8,101 independent sequence contigs, 107 microsatellite loci were detected and primer pairs designed for these. Forty-three of these primer pairs were chosen to be screened and nine of these were reliably amplifiable and polymorphic. These nine markers were genotyped over 618 samples from *Selliera* comprising the three described species.

Populations within all three described species showed high differentiation and *S. radicans* was variable for population structure. Leaf morphological analyses suggested there was a distinct difference between the three species. Microsatellite data revealed two genetic clusters in *S. microphylla* which clustered into the North Island and South Island populations. Two genetic clusters were also observed in *S. rotundifolia* which each clustered with different *S. radicans* populations suggesting

round leaves may have had multiple origins. Hybridization was observed at one sympatric site between *S. radicans* and *S. rotundifolia* and apparent reproductive isolation for *S. rotundifolia* was observed at another site.

These results suggest that the South Island *S. microphylla* population may be an inland variant of *S. radicans* which may continue to diverge if it remains isolated, while the North Island populations should retain the *S. microphylla* name due to the $2n$=56 chromosome count, geographic isolation and genetic distinction although this needs further review. There is evidence of reproductive isolation for *S. rotundifolia* at one of the sympatric sites suggesting this is a distinct species but it appears round leaves may have had multiple origins so may not be suitable to describe the species according to the lineage species concept. This study provides insights into the population structure within and between the described species and has identified interesting areas of future study.

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## ABBREVIATIONS

| | |
|---|---|
| %P | - percentage polymorphic loci |
| A | - number of alleles |
| AMOVA | - Analysis of molecular variance |
| ANOVA | - Analysis of variance |
| bp | - base pairs |
| cm | - centimeters |
| CTAB | - cetyltrimethylammonium bromide |
| d.f | - degrees of freedom |
| DNA | - deoxyribonucleic acid |
| dNTP | - deoxyribonucleotide triphosphate |
| $F_{IS}$ | - factor in Sewell Wrights F statistic. $F_{IS}$ is the inbreeding coefficient that is the proportion of the variance in the subpopulation within an individual. |
| FIJI | - FIJI is just imageJ (image analysis software) |
| $F_{ST}$ | - factor in Sewell Wrights F statistic. $F_{ST}$ is the proportion of the total genetic variance in the subpopulation contained in an individual. |
| $G_{ST}$ | - coefficient of genetic differentiation. Defined as the expected heterozygosity for the total population minus the expected heterozygosity within the subpopulations divided by the expected heterozygosity for the total population. |
| Ho | - observed heterozygosity |
| He | - expected heterozygosity |
| $H_T$ | - species-wide expected heterozygosity |
| ISSR | - inter simple sequence repeat |
| ITS | - internal transcribed spacer |
| *n* | – chromosome number in a haploid |
| N | - number of individuals |
| NA | - number of alleles |
| Ne | - number of effective alleles |
| NZGL | - New Zealand Genomic Limited |

| | |
|---|---|
| P value | - probability of obtaining a test statistic at least as extreme as the one observed assuming the null hypothesis is true |
| PCR | – polymerase chain reaction |
| RNA | - ribonucleic acid |
| Rxy | - correlation coefficient of Mantel test |
| SNP | - single nucleotide polymorphism |
| STE | - sucrose, tris, EDTA |
| *Ta* | - melting temperature |
| TE | - Tris-EDTA buffer |

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