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RESEARCH

Genetic diversity and hybridization in New Zealand's endemic *Lophomyrtus bullata* (Myrtaceae): implications for conservation under myrtle rust threat

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Abstract

The conservation of threatened endemic species is vital to preserving regional biodiversity. *Lophomyrtus bullata* (Myrtaceae), also known as ramarama, is an endemic woody shrub or small tree species threatened by the arrival of the pathogenic fungus *Austropuccinia psidii* (myrtle rust) in New Zealand. The high susceptibility of *L. bullata* to myrtle rust has resulted in populations declining at an alarming rate, likely reducing genetic diversity and potentially increasing its extinction risk. To assess the current levels and distribution of genetic variation in *L. bullata*, twelve microsatellite markers were developed and screened across 18 locations. In addition, one site of the congener, *L. obcordata* (rōhutu), and two putative *L. bullata* x *L. obcordata* hybrid populations were also included in the analysis. Across populations of *L. bullata*, a total of 97 alleles were detected, with few private alleles. A moderately low expected heterozygosity (He = 0.44) and low genetic differentiation among locations (F_{ST} = 0.14) were additionally observed. Individuals were found to best cluster into five distinct genetic groups, largely corresponding to geographic regions. Among the strongest patterns observed was a north-south grade that coincides with a biogeographic pattern known as the Taupō line. In addition to providing insights into the evolutionary history of ramarama, these results also provide a practical guide for seed bank collections, population management, and re-vegetation projects, all of which will help contribute to the long-term conservation of *L. bullata*.

Keywords Lophomyrtus · Myrtaceae · Ramarama · Rōhutu · Biogeography · Population genetics · Myrtle rust · Hybridization

Introduction

Understanding the genetic diversity and population structure of species is a key aspect of conservation biology (DeWoody et al. 2021; Kardos et al. 2021). The level of genetic diversity contributes to the potential of a species to adapt to environmental changes, with low diversity often associated with inbreeding and reduced fitness (Frankel and Soule 1981; Hedrick and Kalinowski 2000; Charlesworth and Willis 2009; Kardos et al. 2021). How genetic variation is distributed across a species' geographic range often reflects connectivity among populations, and the degree of gene flow, which can also be used to make inferences about life history (Hamrick and Godt 1996; Nybom 2004; Aguilar et al. 2008). This information can be readily applied to conservation practices, including identifying high-priority sites for conservation, determining the need for and appropriate scale of ecosourcing in restoration projects, and ensuring germplasm collections are made from viable populations that represent the entire range of regional diversity present within a species (Frankel and Soule 1981; Schoen and Brown 2001). Application of such information has, of course, contributed to the conservation of many threatened species globally (e.g., Maki 2003; Coates et al. 2015; Szczecinska et al. 2016; Hindrikson et al. 2017), as well as to the conservation of several New Zealand species (e.g., Young et al. 2001; Broadhurst et al. 2008; Barnaud and Houliston 2010; Smissen et al. 2011).

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The New Zealand flora has a high frequency (approximately 83–85%) of endemic vascular plant species (McGlone et al. 2001; Millar et al. 2017) with a rich and complex history. Many New Zealand plant species have survived widescale geological changes, including glaciation, and volcanism, resulting in severe population bottlenecks and often subsequent radiations (Fleming 1979; Lockhart et al. 2001; McGlone et al. 2001; Meudt et al. 2015). More recently, endemic species have been impacted by the introduction of exotic pests and diseases and human-mediated habitat destruction and fragmentation (Craig et al. 2000; Beggs 2001; Bradshaw et al. 2020). This has contributed to approximately 45% of the New Zealand indigenous vascular flora being listed as 'at-risk' or 'threatened' under the NZ classification system (de Lange et al. 2018). The potential extinction of these at-risk species would contribute to a global loss of biodiversity and would disrupt ecosystem services and functions on which many species, including humans, depend (Daily 1997; Balvanera et al. 2006; Cadotte et al. 2008; Quijas et al. 2010; Ceballos et al. 2017). Therefore, protecting and conserving New Zealand's endemic plant species is a critical focus.

The current level of biological invasions caused by the introduction of exotic species is unprecedented in Earth's history (Ricciardi 2007) and is a significant contributor to the ongoing extinction crisis (Pyšek et al. 2020). Increased movement of people, animals, wood, and live plants has facilitated the spread of exotic species to new environments (Ridley et al. 2000; Kueffer 2017). These invasions can have severe consequences for native biota, including a decline in genetic diversity within species, altering species richness and community structures (Suarez and Tsutsui 2008; Blackburn et al. 2019; Pyšek et al. 2020), and extinction. In particular, the introduction of invasive microorganisms into new environments has caused major biodiversity losses worldwide (Brasier and Buck 2001; Rigling and Prospero 2018; Scheele et al. 2019), with specific concern being raised about the increasing threat of invasive fungal species (Fisher et al. 2012, 2020). In New Zealand, such a threat already has been realised with the effects of the pathogenic fungus responsible for Kauri dieback disease (*Phytophthora agathidicida*), which causes tree mortality and reduced population numbers of the endemic host species *Agathis australis* (Araucariaceae) (Bradshaw et al. 2020). More recently, the establishment of a new fungal disease, myrtle rust, has the potential to cause similar, if not more widespread effects on the New Zealand flora.

Myrtle rust, also known as guava rust or eucalypt rust, is a disease caused by the pathogenic fungus *Austropuccinia psidii* (Sphaerophragmiaceae), which exclusively affects members of the Myrtaceae family (Glen et al. 2007). The fungus was first described by Winter (1884) in South America, where it infected the local guava (*Psidium guajava*). To date, a total of nine different strains have been identified, with the strain known as the 'pandemic biotype' being the most widespread (Sandhu et al. 2016; Stewart et al. 2018). The highly invasive pathogen has spread globally, including to the USA, Japan, China, South Africa, and Myrtaceae-rich Australia, where it has caused extensive damage at both the species and ecosystem levels, with many endemics at severe risk of localised extinction (Carnegie et al. 2016; Pegg et al. 2017; Fernandez-Winzer et al. 2020). There are limited effective treatments for myrtle rust infections (Pathan et al. 2020) and susceptibility and resistance to the disease vary widely among species and across geographic locations within the same species (Yong et al. 2019).

In New Zealand, the Myrtaceae consists of four tribes (Myrteae, Leptospermeae, Syzygieae, and Metrosidereae), comprising 28 species (Allan 1961; de Lange 2014) although this number will decrease if the most recent treatment of *Kunzea* is recognised (Heenan et al. 2022). Myrtaceae species occur in diverse forest types, with many serving important roles in the early succession of natural and regenerating forests (Wardle 1991; Wiser et al. 2011; Wiser and De Cáceres 2013). Several species are economically important; for example, *Leptospermum scoparium* (mānuka) is used in the production of mānuka honey (Clearwater et al. 2021). Many Myrtaceae also hold cultural significance to the indigenous people of Aotearoa, including *Metrosideros excelsa* (Pōhutukawa), which is highly valued by Māori (Hiroa 1949).

Myrtle rust was detected in New Zealand for the first time in May 2017, potentially arriving via wind currents from Australia (Beresford et al. 2018; Du Plessis et al. 2019; Ho et al. 2019). The pathogen has since spread throughout the North and South Island, infecting 17 native species, resulting in the conservation status of all native Myrtaceae species being elevated to 'threatened – nationally critical' (de Lange et al. 2018). Among the hardest hit by the disease are species of *Lophomyrtus* (Beresford et al. 2020; Sutherland et al. 2020). One of the species, *Lophomyrtus bullata* (ramarama), has been observed to be highly susceptible to the disease, with no perceived natural resistance present (Smith et al. 2020; Sutherland et al. 2020; Toome-Heller et al. 2020). The extent of infection in *L. bullata* is severe, with the leaves, stems, developing fruit, and regenerating seedlings of the species all heavily affected. This suggests there is potential for myrtle rust to cause the localised extinction of *L. bullata* (Sutherland et al. 2020; Toome-Heller et al. 2020), with the death of mature trees already reported (Beyond-Myrtle-Rust 2020). The situation suggests an urgent need for detailed research into both the ecological and genetic aspects of *L. bullata* to support its conservation.

The conservation status of the endemic *L. bullata* was elevated to 'threatened – nationally critical' following the

establishment of myrtle rust (Beresford et al. 2018; de Lange et al. 2018; Du Plessis et al. 2019; Ho et al. 2019). *Lophomyrtus bullata* is a perennial shrub or small tree species with characteristic bullate or 'bubble-like' leaves that are green with an occasional red pigmentation and flowers with five white petals and numerous stamens (Allan 1961). The species is insect pollinated (mainly by bees), and the seeds are encased in red berries that are bird dispersed (Allan 1961; Kelly et al. 2010; Westphal 2019). Traditionally, Māori used the fruits of ramarama as a food source and the leaves for medicinal purposes (Best 1942; Riley 1994; Cambie and Ferguson 2003). The wood of *L. bullata* was traditionally used to make poles by canoeists of the Whanganui tribes and was also used to craft humming tops (Best 1925a, b).

Lophomyrtus bullata is distributed across the North Island and the northeastern corner of the South Island (Fig. 1), generally found in lowland podocarp forests, where it forms an integral part of the forest understory (Cheeseman 1906; Allan 1961; Jo et al. 2022). The species typically occurs on the margins of forests or in canopy gaps, as they tend to prefer open, well-lit areas (Salmon 1980); however, they also can grow in denser forests and wetlands and form an essential part of successional regenerating shrubland (de Lange 2022). Its only congener, *L. obcordata* (rōhutu), another New Zealand endemic shrub, is easily distinguished from *L. bullata* by its smaller, obcordate, non-bullate leaves (Allan 1961). *L. obcordata* has a wider, more patchy distribution across both the North Island and South Island, occurring in coastal and lowland forested habitats, but extending further south and slightly higher in elevation. Where the two species co-occur, they often hybridize, sometimes forming extensive hybrid swarms (Cockayne 1918), which can display near continuous morphological variation from one species to the other (Allan 1929). Reflecting a taxonomic

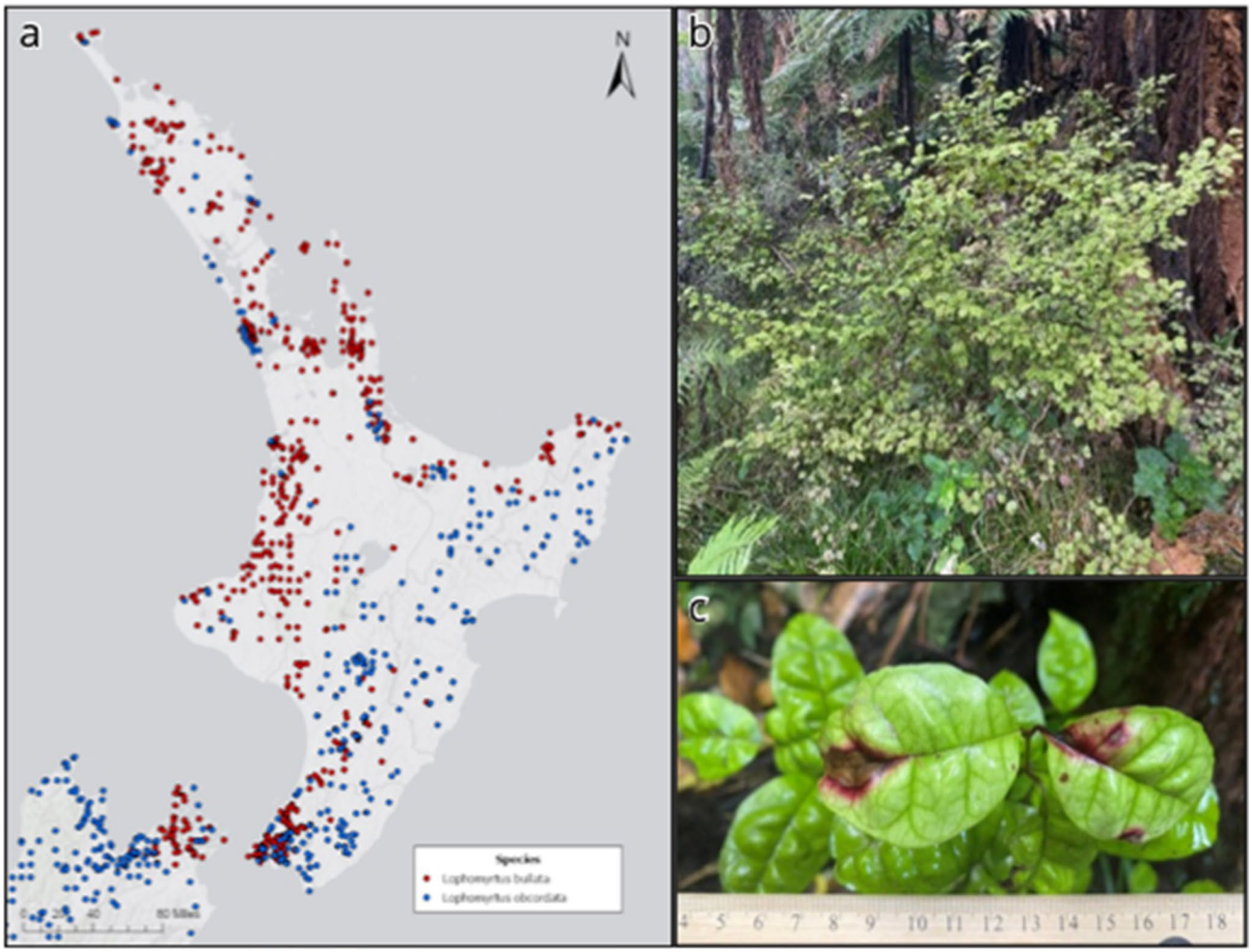


Fig. 1 Geographic distribution of *Lophomyrtus bullata* and *L. obcordata* across the North Island and top of the South Island, and field images of *L. bullata*. (a) Map of the North Island and top of the South Island, showing distribution records from the New Zealand National Vegetation Survey (NVS), iNaturalist, and the Australasian Virtual Herbarium (AVH): red dots indicate occurrences of *L. bullata*, and blue dots indicate occurrences of *L. obcordata*. (b) A *L. bullata* individual in a natural population. (c) *L. bullata* plant showing symptoms of myrtle rust infection, with conspicuous lesions on young leaves.

history where the hybrids were once recognised as a separate species (*Myrtus Ralphii* Hook. f.), the hybrids are now commonly referred to as *Lophomyrtus* x*ralphii*, as proposed by Cockayne (1918). Like *L. bullata*, *L. obcordata* and the hybrids are similarly threatened by myrtle rust (de Lange et al. 2018; Beresford et al. 2020).

There is currently no information on the genetic diversity or population structure of *Lophomyrtus bullata.* To address this knowledge gap and to support conservation efforts, polymorphic microsatellite loci were developed to analyse plants from across the natural geographic range of the species. To assess potential interspecific hybridization and gene flow, *L. obcordata* individuals from one location and putative *L. bullata* x *L. obcordata* individuals from two locations also were included in the analyses. The patterns observed within *L. bullata* are used to investigate biogeographic influences on the distribution of genetic variation within the species and should inform and support the future conservation management of ramarama.

Materials and methods

Sample collection

Potential sampling locations for *L. bullata* and *L. obcordata* were identified using data from the New Zealand National Vegetation Survey database, iNaturalist research grade observations, and the Australian Virtual Herbarium visualised using ArcGIS Pro v3.4 (Fig. 1). A total of 406 individuals of *L. bullata* were collected from 18 sites covering most of the geographic distribution of the species (Fig. 2). From each site, 20–25 individuals were sampled, with an average of 21. A further 20 individuals were collected from each of two sites with suspected *L. bullata* x *L. obcordata* hybrids, and an additional 20 individuals (12 were used in the current study) of *L. obcordata* were collected from a South Island site. To avoid repeated sampling of the same individual and to ensure an unbiased representation of each population, individuals were randomly sampled along a transect within each site. The spacing of sampled individuals varied to accommodate both the site and population size. For each individual, two to three leaves were collected and placed in zip-lock plastic bags containing silica gel to desiccate and preserve the tissue until further use. A photo voucher for each population was made and deposited in the Dame Ella Campbell (MPN) Herbarium, and GPS coordinates and other relevant observations were noted. Prior to further processing, all samples were left to dry in silica gel for at least one week following field collection.

Collections were made between March and October 2022. The collection sites included Department of Conservation (DoC) land, privately and iwi-owned land, and land managed by the Auckland Regional Council (Fig. 2). Approvals for sampling were obtained through the Manaaki Whenua-Landcare Research, Beyond Myrtle Rust program and was granted by the owner or iwi for privately or iwi-owned land. For DoC reserve sites, permission was granted utilising the Manaaki Whenua-Landcare Research Global concession (permit no. CA-31615-OTH). Local iwi were contacted for each site, with consultation and approval sought for sampling.

During field work, precautions were taken to limit the spread of myrtle rust between different sites and individuals within the same site. These precautions included: the wearing of overalls and hats that were removed and bagged before leaving each site and washed between site visits, all equipment and footwear were cleaned after use at each site with 70% ethanol (or isopropyl alcohol), and gloves were worn when collecting on site and cleaned with 70% ethanol (or isopropyl alcohol) between individual collections. Great care was also taken to avoid the collection of infected material. The approximate population size and associated flora were also noted for each site, as well as any evidence of either current or past infection of myrtle rust.

Microsatellite marker development, screening, and analysis

From one sample of *L. bullata* collected on the Massey University Manawatū campus (Palmerston North, New Zealand), a random sample of genomic DNA sequences were obtained via an Illumina MiSeq run. Young fresh leaf tissue was collected and genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1987). The genomic DNA was then sheared into 200–500 bp sections using a sonicator, after which Illumina sequencing adapters and individual-specific sequence tags were ligated to the ends for library construction. Sequencing was then carried out on one-half of a 2 × 250 bp paired-end MiSeq run (Illumina), resulting in 8,815,516 raw reads.

The reads were paired and merged using the GENEIOUS software (version 9.1.8) (Kearse et al. 2012), with 1,351,112 of these sequences successfully merged. The sequences were then uploaded as a Fastq file to the Galaxy web platform (Community 2024) and repeat regions were identified using the STR detection tool (Fungtammasan et al. 2015). The parameters for repeat detection were as follows: a minimum length of seven uninterrupted dinucleotide repeats and a 50 bp buffer on each side of the repeat regions. Partial motifs were not considered, and a hamming threshold of zero was applied. From the search, 22,742 dinucleotide repeats were detected, ranging from 7 to 104 repeat units.

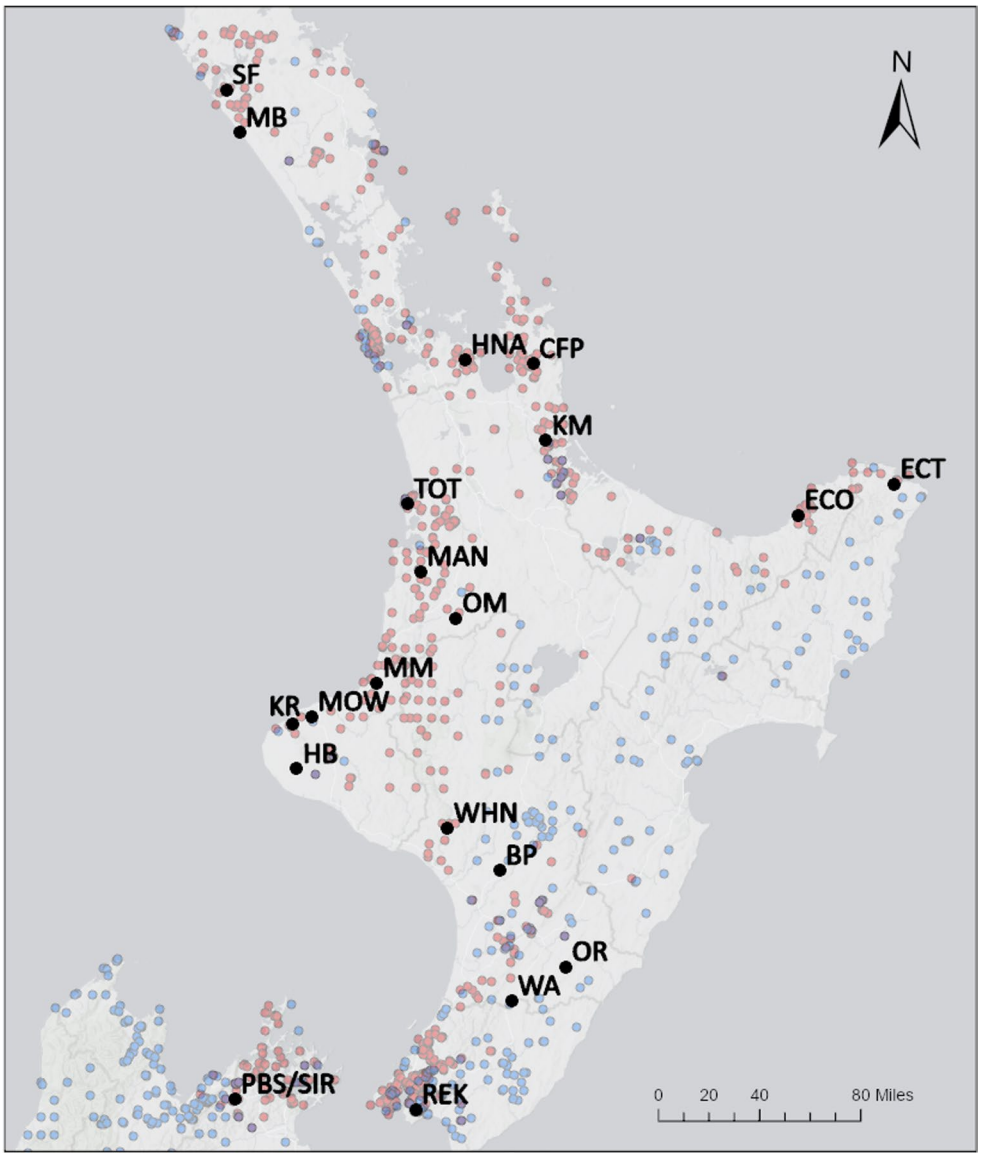


Fig. 2 Sampling locations for *Lophomyrtus bullata*, *L. obcordata*, and putative hybrids, shown in relation to species occurrence records. Each black circle marks the GPS coordinates of a sampling site. The single *L. obcordata* sampling site is SIR and the two sites with morphologically intermediate individuals are MOW and WA. Red circles indicate observational records of *L. bullata*, blue circles indicate *L. obcordata*, and purple circles represent putative hybrid locations.

A range of microsatellite loci that differed in repeat length (from 7 up to 11) were selected, and the GENEIOUS add-in PRIMER3 (Rozen and Skaletsky 2000) was used to design flanking primers. The criteria used to design the primers included a product size range of 120–300 bp, a primer length of 17–21 (bp) with 19 selected as the optimum, a melting temperature between 52–58 °C, a 50% GC content and a GC clamp of at least one. Primer pairs were discounted if more than 5 bp mononucleotide repeats were present within the region between the primers and the microsatellite locus or within the primer region itself. A 19 bp M13 tail sequence was added to the 5' end of forward primers (Boutin-Ganache et al. 2001), and a 'pigtail' sequence was added to the 5' end of reverse primers (Brownstein et al. 1996).

In total, 55 primers were designed, and through an initial screen of eight individuals of *L. bullata* from a Wellington population, 36 primer pairs were identified that amplified well as assessed on a 1.5% agarose gel stained with ethidium bromide. These 36 primer pairs were then screened on 15 individuals of *L. bullata* from five different sites: Northland, Tauranga, Taranaki, Te Kuiti, and Wellington.

The PCR products were then checked on a 1.5% agarose gel. For loci that amplified well, 3 µL of the PCR products from four different loci were pooled, and 1.25 µL of that pooled solution was added to 9 µL of Hi-Di formamide (Applied Biosystems) with CASS size standard (Symonds and Lloyd 2004). The samples were separated and sized by capillary electrophoresis on an Applied Biosystems 3130XL genotype analyser at the Massey Genome Service Centre (Palmerston North, New Zealand). Allele scoring was completed using GENEMAPPER v.4.0 (Applied Biosystems).

Of these 36 markers, several were discounted because they were monomorphic or showed evidence of amplification of

more than one locus. Twelve of the 36 markers were selected for subsequent trialling based on consistent and clean amplification and relatively moderate levels of variability. Using the above methods, the markers were then trialled on a further 24 individuals from four populations: Northland, Auckland, Taranaki, and Wellington. The final 12 microsatellite markers were also checked for cross-amplification in the closely related species *Lophomyrtus obcordata*, using 12 individuals from a South Island population. All remaining individuals were screened for these 12 microsatellite loci following the same PCR and genotyping methods described above.

Following a second round of genotyping to fill in missing data, individuals with missing data for more than three loci (six individuals) were removed from the dataset, leaving a total of 452 individuals for further analysis. The data were formatted for GenAlEx software (version 6.5) (Peakall and Smouse 2006, 2012) and checked for null alleles and possible sequencing errors using MICRO-CHECKER software v.2.3.3 (Van Oosterhout et al. 2004). For all following analyses, the dataset was split into two datasets; dataset *L.bull* which contained data for only the 18 *L. bullata* sites, and dataset *L.all*, which contained the same 18 *L. bullata* sites in addition to the two putative hybrid sites (Meeting of the Waters and WA Miller Memorial Park), and twelve individuals of *L. obcordata* from the one South Island location. This partitioning allowed for separate analyses to be completed on the data both with and without the influence of hybridisation. The number of alleles, number of effective alleles, number of private alleles, observed and expected heterozygosity, Wright's fixation indices (Wright, 1931), deviations from Hardy-Weinberg Equilibrium (HWE), and pairwise F_{ST} distance matrices were all calculated using GenAlEx. Regression analyses between expected heterozygosity and latitude and the number of alleles per locus (Na) and latitude were additionally calculated in Excel.

The datasets were then exported separately into STRUCTURE (Pritchard et al. 2000) to identify genetic groups. The parameters used for each analysis were: 150,000 iterations of burn-in and 1,000,000 Monte Carlo Markov Chain iterations, with the admixture model used and allele frequencies set to correlated. $K = 1–10$ were interrogated with ten replicate runs for each K value. The resulting output files were uploaded to STRUCTURE HARVESTER v.0.6.94 (Earl and VonHoldt 2012) to test for the mean likelihood of K values using the Evanno method (Evanno et al. 2005). The STRUCTURE results were additionally uploaded to CLUMPAK (Kopelman et al. 2015), which uses a Markov clustering algorithm to cluster replicate runs for the same K value. This allowed for the identification of the optimal alignment of the replicate runs for each value of K and for comparisons between different K values to be made.

For further analyses, the two datasets were separately imported into R studio (RStudio-Team, 2020). The *provesti.dist* function from the R package *poppr* (Kamvar et al. 2014) was employed to generate a population-level genetic distance matrix. The distance matrices were then separately converted into the Nexus format and exported to SPLITSTREE5 (Huson and Bryant 2006) to create a population-level Neighbor-Net splits graph.

The R package *adegenet* was used to analyze the datasets using Discriminant Analysis of Principal Components (DAPC) (Jombart et al. 2010). The *find.clusters* function (Jombart 2008) was used first to transform the data into principal components, then cluster the data into a specified number of groups. The Bayesian information criterion (BIC) identified a K of ~7 as the most supported number of groups for the dataset *L.bull* and a K of ~12 for the dataset *L.all*. However, K-means of 2–12 for both datasets were investigated and were also visualised utilising the *compoplot* function. For all DAPC analyses, 40 Principal Components (PCs) were retained, and the first two were plotted.

Due to the continuous nature of the genetic variation present in these datasets, the clustering algorithm of DAPC was attempting to identify groups that are structured in a gradient. This results in groupings that contain a large degree of overlap, which makes it difficult to interpret. The authors of the software acknowledge such problems with continuous data (Jombart et al. 2010), and suggest using the scatterplot function to visualise results. This allows the interpretation of any genetic structure across such gradients (Jombart et al. 2010). This method of visualisation was applied to these datasets.

To further account for potential clinal patterns in genetic structure, we used the TESS3 algorithm implemented in the *tess3r* R package (Caye et al. 2016). Unlike STRUCTURE, which assumes discrete populations, TESS3 incorporates spatial information to better detect continuous or gradient-like variation in ancestry. The microsatellite genotypes were converted from genind to the required *tess3r* input format. Geographic coordinates were provided for each individual, and ancestry coefficients were estimated across a range of K values (2–10). Cross-entropy scores were used to assess model fit and guide selection of the most informative number of ancestral clusters. Ancestry coefficients were visualised with interpolated barplots and spatial maps to examine spatial genetic gradients. As TESS3 was utilised to assess the apparent clinal gradient in *L. bullata*, only the *L.bull* dataset was examined and the putative hybrid and *L. obcordata* samples were excluded from these analyses.

An AMOVA test was performed in GenAlEx (version 6.5) to determine how variation is partitioned within and among populations. The test was completed using 999 permutations and was performed on both datasets, *L.bull* and

L.all. Only dataset *L.bull* was used to test for isolation by distance via the Mantel test due to Meeting of the Waters and WA Miller Memorial Park populations containing putative hybrids. For the Mantel test, GENEPOP software v.4.7.5 (Raymond and Rousset 1995) was used to transform pairwise F_{ST} values into $F_{ST}(1\text{-}F_{ST})$ (Rousset 1997), which was tested against the geographical distance and the natural log of geographical distance in GenAlEx.

To assess whether populations have experienced genetic bottlenecks, the software BOTTLENECK v.1.2.02 (Cornuet and Luikart 1996) was used under the TPM model, assuming 70% SMM. This approach looks for signs of a recent reduction in effective population size. Populations were assessed using a Wilcoxon signed-rank, one-tailed test, where a statistically significant result was taken as evidence for a recent bottleneck.

Results

MICRO-CHECKER identified potential null alleles at one locus (LB47), though this is due to an excess of homozygosity, which could be due to reasons other than the presence of null alleles. Most loci showed significant deviations from HWE for at least one population due to heterozygosity deficiency; however, the data were unmodified for subsequent analyses, as these deviations were likely due to natural biological processes and removal of loci would decrease the resolution of genetic structure, impacting the interpretation of results (Dharmarajan et al. 2013).

Genetic diversity

The summary statistics for each population are reported in Table 1. Across all samples and sites, the one *L. obcordata* site (SIR) had the greatest number of private alleles with nine (Table 1), which is unsurprising as it was being compared with populations of a different species. Among the *L. bullata* sites, the greatest number of private alleles found was three at the northern-most SF site, which also had the greatest expected heterozygosity (0.57). The analysis comparing expected heterozygosity and latitude for the *L. bullata* populations showed a strong correlation, with He decreasing from north to south ($P = 0.00002$, R^2: 0.72). The analysis comparing the number of alleles per locus and latitude showed a similar correlation, with the number of alleles per locus also decreasing from north to south ($P = 0.00002$, R^2: 0.66). BOTTLENECK tests indicated that four populations had likely experienced recent genetic bottlenecks (data not shown): an East Cape population (ECT), a Whanganui population (WHN), and the two putative hybrid sites (MOW and WA).

Table 1 Population genetic metrics for *L. bullata, L. obcordata*, and putative hybrid sites. Column labels are: number of samples (N), number of alleles (NA), number of effective alleles (Ne), number of private alleles (PA), percentage of polymorphic loci (%P), observed heterozygosity (Ho), expected heterozygosity (He), and the fixation index (F_{IS})

Site	Site Code	*N*	Na	Ne	Pa	%*P*	Ho	He	F_{IS}
Six Foot Track	SF	22	4.42	2.75	3	100	0.56	0.57	0.01
Maunganui Bluff	MB	18	4.33	2.80	2	100	0.46	0.53	0.07
Hunua Park	HNA	22	4.42	2.30	3	100	0.51	0.47	−0.06
Coromandel Park	CFP	22	3.92	2.54	2	100	0.49	0.52	0.04
Kaimai Mamaku	KM	22	3.67	2.23	0	100	0.48	0.48	−0.01
East Cape One	ECO	20	3.17	2.37	2	83.33	0.47	0.49	0.04
East Cape Two	ECT	20	3.00	1.97	0	83.33	0.38	0.40	0.08
Te Toto Gorge	TOT	24	3.8	2.11	1	100	0.42	0.43	0.03
Mangapohue	MAN	23	3.92	2.27	1	100	0.51	0.47	−0.05
Omaru Falls	OM	24	3.10	2.06	0	100	0.40	0.42	0.05
Mt Messenger	MM	22	2.83	1.88	0	83.33	0.42	0.39	−0.08
Meeting of the Waters	MOW	20	3.92	2.71	1	100	0.54	0.56	0.03
Kaitake Ranges	KR	24	2.67	1.85	0	75	0.35	0.37	0.06
Home Bush	HB	24	3.00	2.05	0	91.67	0.4	0.43	0.04
Whanganui Park	WHN	23	2.75	1.94	0	83.33	0.41	0.42	0.04
Bruce Park	BP	24	3.25	2.06	1	83.33	0.42	0.42	0.02
Ohinerata	OR	24	2.83	1.75	1	83.33	0.38	0.36	−0.03
WA Miller Park	WA	20	2.92	2.28	0	91.67	0.59	0.51	−0.16
Remutaka Park	REK	23	2.83	1.90	1	83.33	0.38	0.38	−0.01
Pelorus Bridge	PBS	19	2.92	1.91	1	91.67	0.38	0.39	0.07
South Island Rōhutu	SIR	12	3.83	2.42	9	91.67	0.44	0.42	−0.06

Genetic structure of *Lophomyrtus bullata*

STRUCTURE analyses for the *L.bull* dataset revealed the strongest support for $K=2$ (Fig. 3). While the Evanno method cannot determine a ΔK value for $K=1$, it has been suggested that if a ΔK value for $K=2$ is >53, then there is good support for the solution (Cullingham et al. 2020). At $K=2$, all upper North Island sites (SF, MB, HNA, CFP, KM) and the East Cape sites (ECO, ECT) largely grouped together, as did all sites south of 38°S latitude (MM, KR, HB, WHN, BP, OR, REK, PBS), with the three Waikato populations (TOT, MAN, OM) appearing as a stratified gradient between the North and South groups (Fig. 4a). At $K=3$, the East Cape populations (ECO, ECT) are clustered together in a separate group from the original north and south groupings; however, there is some overlap with the Tauranga population (KM). At $K=4$, the Waikato (TOT, MAN, OM), Taranaki (MM, KR, HB), Whanganui (WHN), and South Island populations (PBS) are clustered together, though this is subdivided at $K=5$ into two groups, one containing all Waikato populations (TOT, MAN, OM), and the other containing the remaining populations. After $K=2$, $K=5$ had the next highest support based on the Evanno method and results for higher K values were unstable. The groupings at $K=5$ generally correspond to the geographic locations of the sites (Fig. 4b); however, there is a high degree of admixture. The TESS3 results mirrored the STRUCTURE results for $K=2$ and $K=3$ and were similar at $K=4$ and $K=5$, with only the associations of the Taranaki and Waikato populations varying (data not shown).

The patterns observed in the population-level Neighbor-Net analyses (Fig. 5) for the dataset *L.bull* are similar to the STRUCTURE results. The largest split observed in the analysis separates the two East Cape populations (ECO, ECT) from the rest of the sites with the next longest split distinguishing the two Northland populations (SF, MB). In each case, the two populations are further separated by long branches, suggesting further divergence within each region. Similar to the STRUCTURE results for $K=2$, the upper North Island sites largely cluster together (SF, MB, CFP, KMW, HNA, ECT, and ECO) and the lower North Island sites and the one South Island site cluster together (MM, HB, WHN, KR, BP, REK, OR, and PBS), with the Waikato sites (TOT, MAN, OM) falling in between.

The scatterplot of the DAPC analysis for $K=7$ shows the same north to south cline as other analyses, with the East Cape (ECO, ECT) individuals showing a moderate degree of separation from the other sites (Fig. 6a). In Fig. 6b, where an orange-to-blue colour gradient is applied based on latitude, the north to south pattern is again very clear.

Genetic structure of *Lophomyrtus bullata, L. obcordata*, and putative hybrids

The pairwise F_{ST} values for the one *L. obcordata* population examined identify significant genetic differentiation from the *L. bullata* populations, ranging from 0.23 to 0.31 (Supplementary Table 1). The highest F_{ST} values are between ECO and SIR (0.31). As expected, the pairwise F_{ST} values for the *L. obcordata* population and the putative

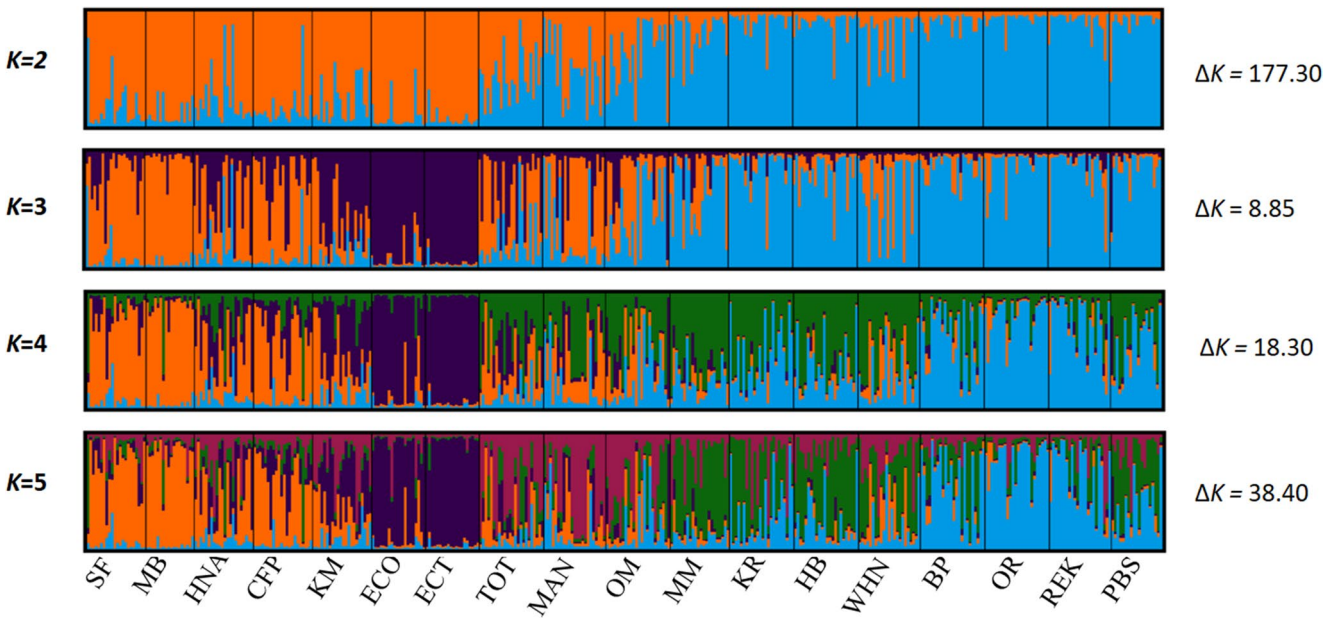


Fig. 3 STRUCTURE plots showing the ancestry coefficient for *L. bullata* individuals. Sites are arranged from north to south and labelled according to site code (along x-axis), K values are shown at left and ΔK values are shown at right. Colors correspond with the genetic groups identified by STRUCTURE.

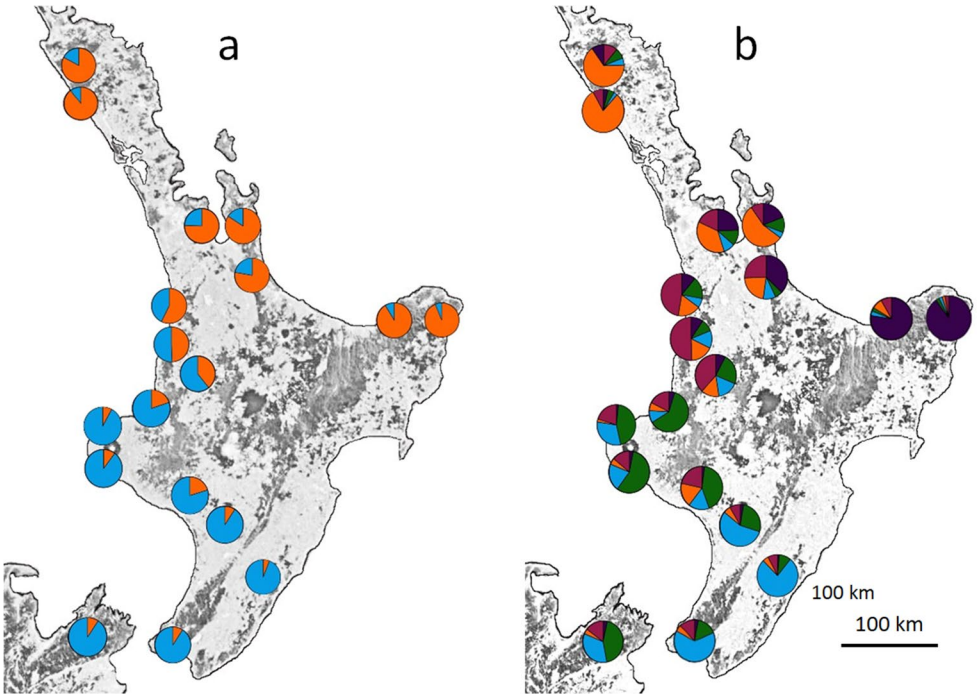


Fig. 4 Map of the North Island and top of the South Island of New Zealand showing the *L. bullata* STRUCTURE results for $K=2$ (a) and $K=5$ (b) plotted by geographic location. Each pie chart represents a sample site, with the colours representing the proportion of that populations' membership to the genetic clusters identified by STRUCTURE.

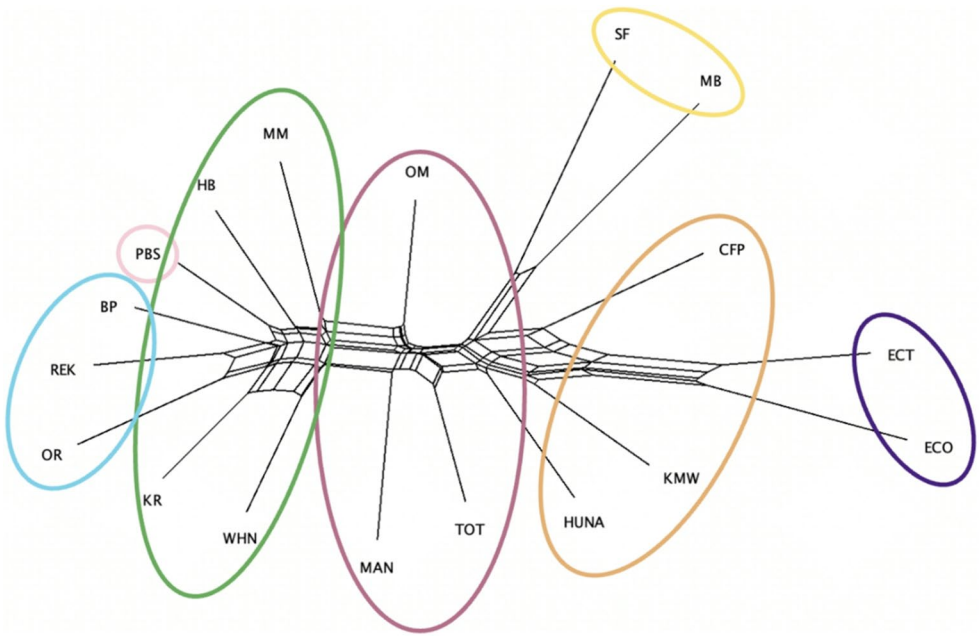


Fig. 5 Population-level Neighbor-Net of *L. bullata* sites. Sites are labelled by site code, with coloured ovals indicating geographic location. Northland: SF, MB (yellow); Auckland/Bay of Plenty: HNA, CFP, KM (orange); East Cape: ECO, ECT (purple); Waikato: TOT, MAN, OM (mauve); Taranaki/Whanganui: MM, KR, HB, WHN (green); Manawatū/Wellington: BP, OR, REK (blue); South Island: PBS (pink).

hybrid populations (MOW, WA) were considerably lower (0.16, 0.18). The overall F_{ST} value for all *L. bullata* populations was roughly the same whether the hybrid sites were included or not ($F_{ST}=\sim 0.14$).

The results of the STRUCTURE analyses for the dataset *L.all* showed highest support for $K=3$, 4, and 6 (Fig. 7). Similar to the *L. bull* results, there were high amounts of admixture among genetic groups. The groups identified for $K=2$ were the same two groups identified for the *L.bull* dataset, with the hybrid sites (MOW, WA) and the *L. obcordata* site (SIR) grouping with the northern *L. bullata* sites (SF, MB, HNA, CFP, KM, TOT, MAN, OM) (Fig. 7); however, the $K=2$ result had relatively little support. At $K=3$, the hybrid (MOW, WA) and *L. obcordata* (SIR) sites are clustered in a separate group that is retained in the results of all higher *K* values. The following *K* values (4–6) split all other populations into roughly the same five groups seen in the *L.bull* results.

In the Neighbor-Net analysis that includes all samples (Fig. 8), the *L. obcordata* site (SIR) and the two putative hybrid

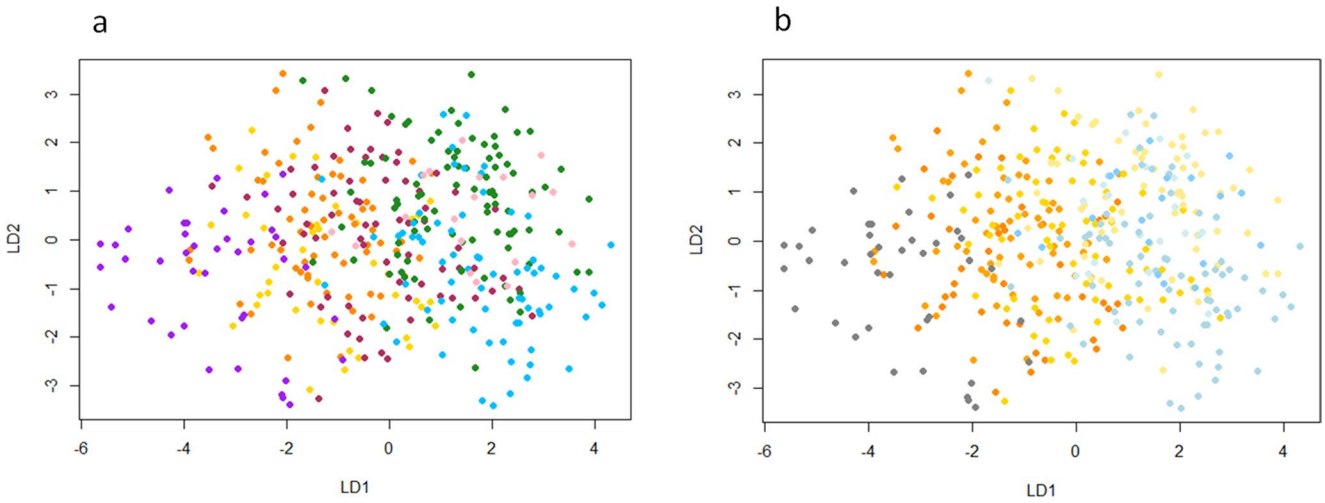


Fig. 6 DAPC scatterplots of *L. bullata* individuals. **a** Individual plot points are coloured to indicate geographic locality as in Fig. 5. **b** Individual plot points are coloured by gradient to indicate positions from north (orange) to south (blue); grey plot points indicate the East Cape populations.

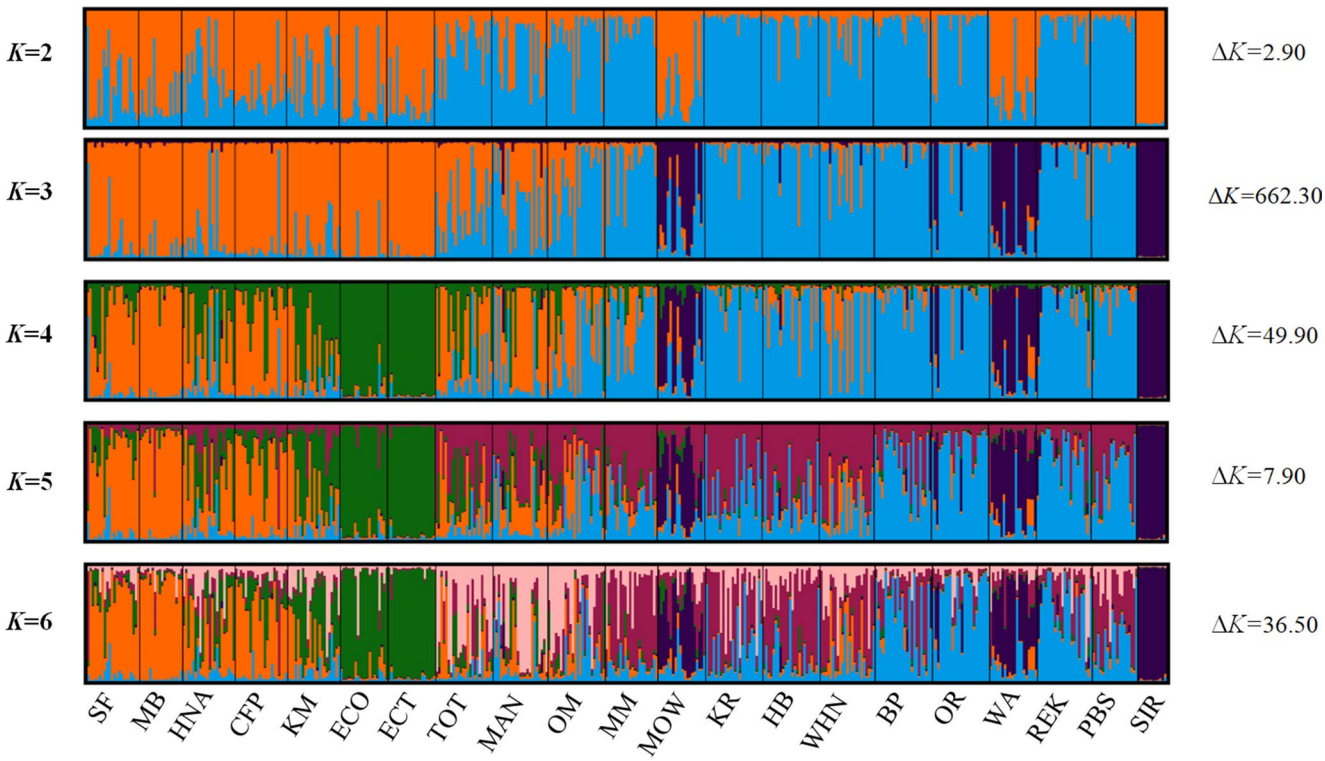


Fig. 7 STRUCTURE plots for *L. bullata*, *L. obcordata* (SIR), and putative hybrid (WA, MOW) sites. Sites are arranged from north to south and labelled according to site code (along x-axis), *K* values are shown at left and Δ*K* values are shown at right. Colours correspond with the genetic groups identified by STRUCTURE.

sites (WA and MOW) are separated by a split from all *L. bullata* sites and the *L. obcordata* site is further differentiated from the two putative hybrid sites. The relationships among the *L. bullata* sites are roughly the same as those shown in Fig. 5. Similar to the Neighbor-Net, the DAPC analysis grouped *L. bullata* individuals from the *L. obcordata* individuals with the two putative hybrid sites positioned in between (Fig. 9).

AMOVA and IBD analyses

AMOVA analysis of dataset *L.bull* at the population-level revealed that 81% of the variation originated within populations, with only 19% variation observed among populations. This pattern was also reflected in the analysis of dataset *L.all*, with 77% of the variation occurring within

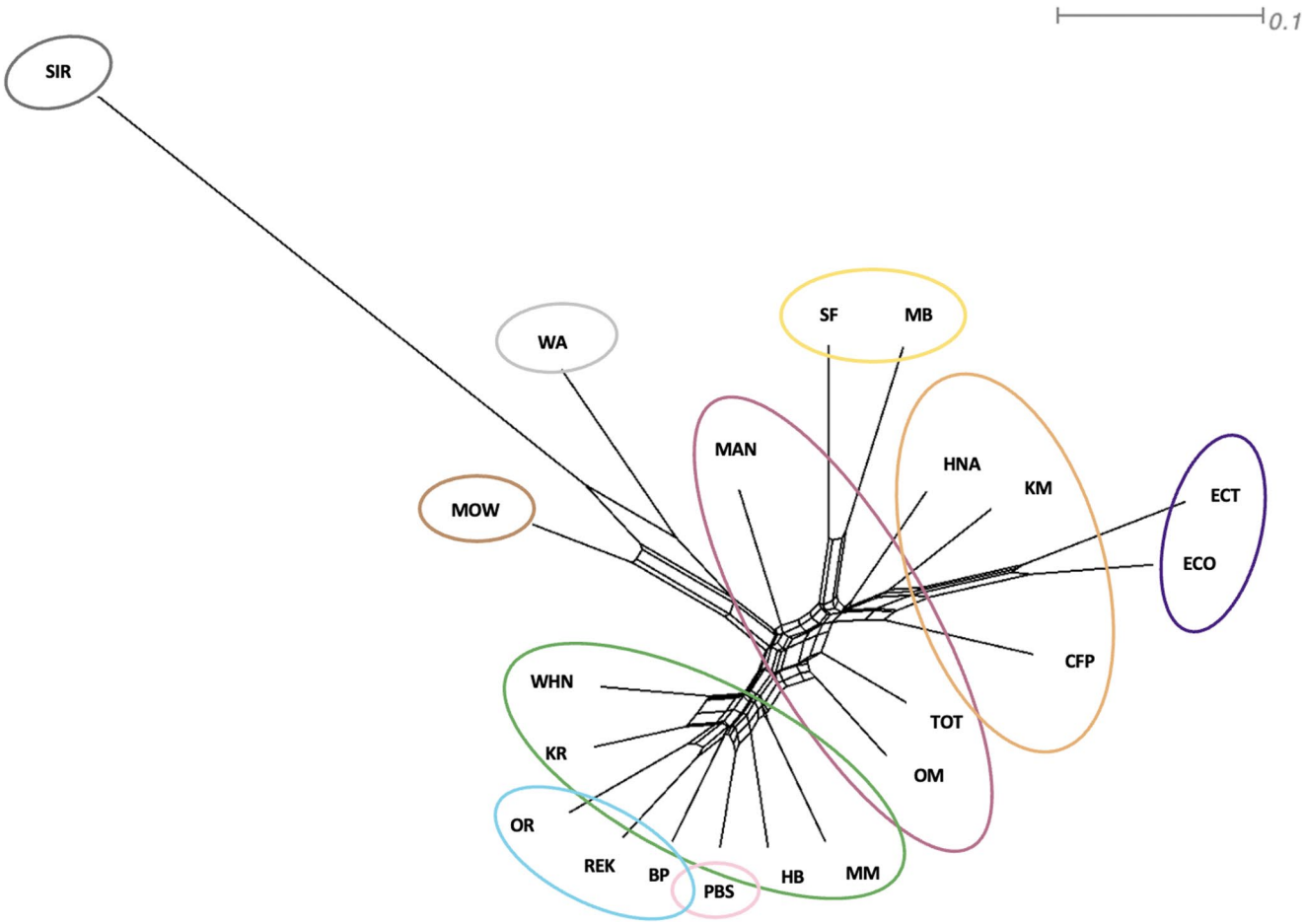


Fig. 8 Population-level Neighbor-Net of *L. bullata*, *L. obcordata*, and putative hybrid sites. Sites are labelled by site code, where coloured ovals indicate geographic locations or putative hybrids. Northland: SF, MB (yellow); Auckland/Bay of Plenty: HNA, CFP, KM (orange); East Cape: ECO, ECT (purple); Waikato: TOT, MAN, OM (mauve); Taranaki/Whanganui: MM, KR, HB, WHN (green); Manawatū/Wellington: BP, OR, REK (blue); South Island (*L. bullata*): PBS (pink); putative hybrid sites: MOW, WA (brown, light grey); *L. obcordata*: SIR (dark grey).

populations and 23% occurring among populations. The Mantel test showed significant isolation by distance for all genetic distance measures (Table 2).

Discussion

Following the arrival of the myrtle rust pathogen in New Zealand, the highly susceptible *Lophomyrtus bullata* was reclassified from 'Not Threatened' to 'Threatened – Nationally Critical' (de Lange et al. 2018). Repeated infections of the fungal disease result in extensive loss of foliage, premature dropping of fruits and, ultimately, plant death (Smith et al. 2020; Sutherland et al. 2020; Toome-Heller et al. 2020). Infected *L. bullata* exhibit all of these symptoms, and regional populations of the species are rapidly declining, indicating strong potential for localised extinction (Smith et al. 2020; Sutherland et al. 2020; Toome-Heller et al. 2020). The regional disappearance of *L. bullata* could further isolate remaining populations, leading to decreased connectivity and gene flow, while rapidly decreasing population sizes would likely increase the effects of genetic drift (Ellstrand and Elam 1993). This could lead to reduced fitness for the species and increase its risk of outright extinction. Insights into the amount of genetic diversity present within *L. bullata* and how that variation is partitioned across its distribution are fundamental to ensuring the best possible conservation management outcomes for the species.

The results from the current study suggest that the genetic diversity of *Lophomyrtus bullata* at a species level is relatively low, with an expected heterozygosity (He) of 0.44 and an average number of alleles per locus of 3.4 (Tables 1 and 3). Comparing these results to those of species with similar life history characteristics in a review of plant genetic diversity by Nybom (2004), *L. bullata* displays a lower He than expected for long-lived perennials (0.68), outcrossing

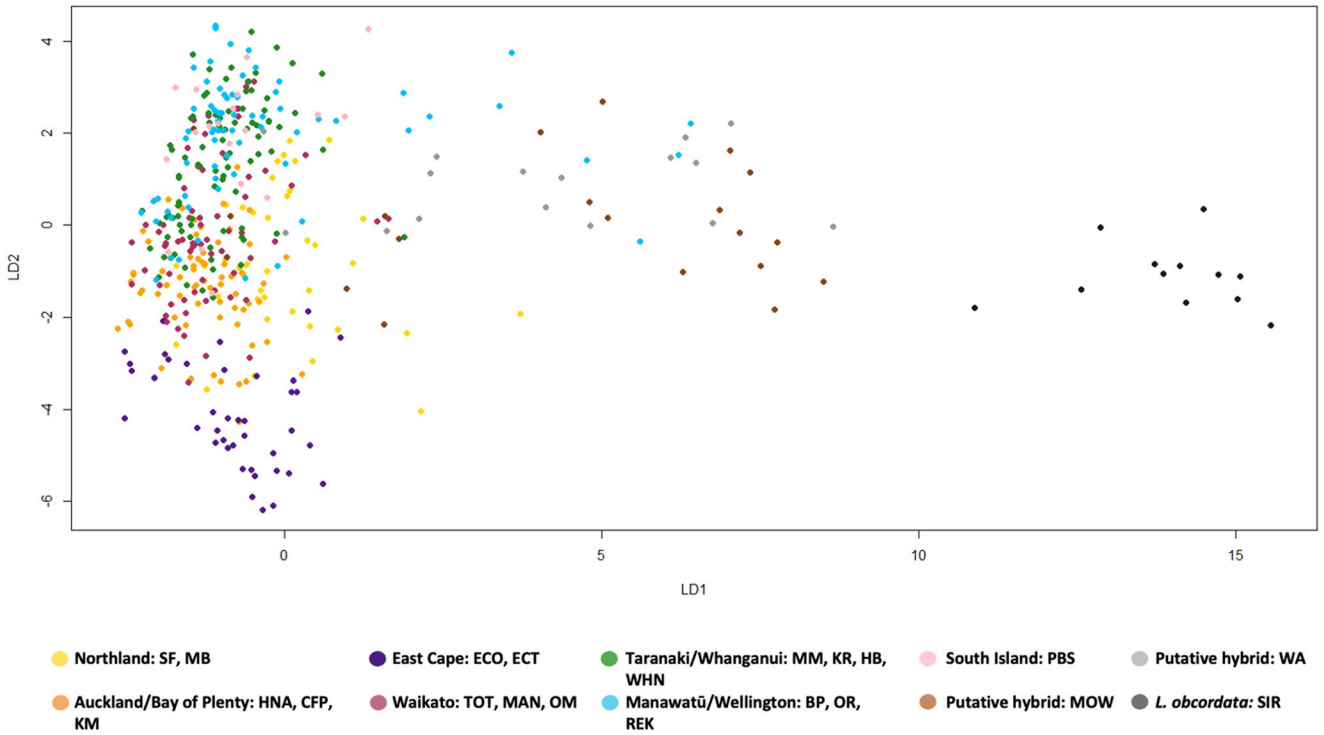


Fig. 9 DAPC scatterplot of *L. bullata*, *L. obcordata*, and the putative *L. bullata* x *L. obcordata* hybrid individuals. Colouration indicates geographic locality of populations as indicated in the key.

Table 2 Mantel test results for populations of *L. bullata*. Rxy: correlation coefficient. P(rxy-rand>=rxy-data): probability of a positive autocorrelation (one-tailed t test)

Genetic Distance metric	Geographical distance (km)	Rxy	*P*(rxy-rand>=rxy-data)
$F_{ST}(1-F_{ST})$	Standard	0.57	0.001
Pairwise F_{ST}	Standard	0.61	0.010
$F_{ST}(1-F_{ST})$	Natural log	0.56	0.010

species (0.65), and species with seeds that are dispersed by ingestion (0.73). Comparisons with other New Zealand species or Myrtaceae species with similar life history traits to *L. bullata* also show that *L. bullata* has lower He (Table 3). For example, the similarly widespread species *Kunzea robusta* has a significantly higher He compared to *L. bullata* (Heenan et al. 2022). While similar levels of genetic diversity were found in *Pseudopanax ferox*, which is widespread around New Zealand, in contrast to *L. bullata*, *P. ferox* populations are highly disjunct, and some contain only a few individuals (Shepherd and Perrie 2011).

One potential source of low within-population genetic diversity would be genetic bottlenecks. An assessment of all sampled *Lophomyrtus* populations identified just four locations with indications of recent genetic bottlenecks. Two of the four locations are the hybrid sites (MOW and WA) and one is the highly admixed WHN site. Because BOTTLENECK analyses seek to identify recent reductions in effective population size by testing for heterozygosity excess relative to that expected under mutation-drift equilibrium (Cornuet and Luikart 1996), these three locations, which contain genotypes from multiple genetic clusters, may show false signals of recent bottlenecks due to admixture rather than true demographic contractions. The fourth site, ECT, showed the strongest signal of a recent genetic bottleneck. Located on the East Cape, this site has been severely impacted by myrtle rust, with the individuals sampled for this study representing the last remnants of a population that once numbered in the hundreds (Graeme Atkins, pers. comm.).

Genetic structure of *Lophomyrtus bullata*: a North-South gradient

Among the best supported patterns observed in the analyses presented here was a north to south partition of genetic variation, with two distinct genetic groups identified in STRUCTURE analyses (Fig. 3) and other analyses. While the most northerly and southerly sites are fairly genetically distinct, the transition is not abrupt. Instead, there is a gradient between the two genetic groups that occurs across locations situated at ~38°- 39°S latitude. This pattern is also evident in the Neighbor-Net analysis, with one of the most prominant splits occurring between the upper North Island and the lower North Island and South Island sites (Fig. 5).

This pattern is consistent with, though somewhat north of a known biogeographic demarcation referred to as the Taupō

Table 3 Population genetic studies of New Zealand native plant species and other myrtaceae species used to compare expected heterozygosity (He) with *Lophomyrtus bullata*. All cited studies were conducted using microsatellite markers

Species	Family	Distribution	Growth form	Breeding system	H_e	Reference
Lophomyrtus bullata	Myrtaceae	Widespread, continuous	Shrub or tree at maturity	Outcrossing	0.44	This paper
Dactylanthus taylorii	Balanophoraceae	Rare, disjunct	Parasite	Outcrossing	0.46	McLay et al. (2022)
Pseudopanax ferox	Araliaceae	Widespread, disjunct	Shrub or tree at maturity	Outcrossing	0.48	Shepherd and Perrie (2011)
Metrosideros bartletti	Myrtaceae	Rare, disjunct	Tree at maturity	Outcrossing	0.50	Melesse (2017)
Pseudopanax crassifolius	Araliaceae	Widespread, continuous	Shrub or tree at maturity	Outcrossing	0.63	Gemmell et al. (2022)
Kunzea ericoides	Myrtaceae	Widespread, continuous	Shrub or tree at maturity	Primarily outcrossing	0.70	Heenan et al. (2022)
Corymbia calophylla	Myrtaceae	Regional, continuous	Tree at maturity	Outcrossing	0.71	Sampson et al. (2018)
Melaleuca alternifolia	Myrtaceae	Regional, disjunct	Shrub or tree at maturity	Outcrossing	0.72	Rossetto et al. (1999)
Kunzea robusta	Myrtaceae	Widespread, continuous	Shrub or tree at maturity	Primarily outcrossing	0.74	Heenan et al. (2022)
Eucalyptus gomphocephala	Myrtaceae	Regional, disjunct	Tree at maturity	Primarily outcrossing	0.75	Nevill et al. (2014)
Metrosideros polymorpha var. glaberrima	Myrtaceae	Widespread, disjunct	Shrub or tree at maturity	Outcrossing	0.76	Stacy et al.(Stacy 2020)
Eucalyptus globulus	Myrtaceae	Widespread, continuous	Tree at maturity	Primarily outcrossing	0.82	Steane et al. (2006)

line. The pattern was originally described by Wardle (1963) as an area that divided the endemic species-rich upper North Island and the species-poor lower North Island. The pattern also has been identified in phylogenetic analyses, with Heenan et al. (2017) identifying a significant decrease in both the phylogenetic diversity and the weighted phylogenetic endemism for the vascular flora in the region south of the Taupō line. This pattern is evident in invertebrates, and vertebrates, with higher levels of both inter- and intraspecific diversity reported for a variety of endemic species in the north (e.g., Buckley et al. 2009; Chapple et al. 2009; Buckley et al. 2010; Marske et al. 2011; Bulgarella et al. 2014; Painting et al. 2017; Withers et al. 2021) as well as in plant species (Heenan et al. 2022).

The precise location and cause(s) of the trend around the Taupō line have been debated (Buckley et al. 2015; Ellis et al. 2015). Many postulate that the formation of a sea strait during the Pliocene, caused by the combination of tectonic uplift and rising sea levels, is the source of the division (Suggate 1978; Bunce et al. 2009). Due to the formation of this sea strait (also known as the Manawatū Sea Strait), flora and fauna below 38° on the North Island would have faced near extirpation (Buckley et al. 2015; Marske and Boyer 2022). Others suggest Pleistocene glacial cycling as the main cause. Glacial cycling during the Last Glacial Maximum (LGM) heavily impacted the structure and range of the forests around New Zealand (McGlone et al. 2010; Newnham et al. 2013; Wood et al. 2017). Formerly widespread forest habitats contracted in size due to glaciation and changes in climate would have restricted many species to glacial refugia. In New Zealand, regions north of the Bay of Plenty often have been proposed as glacial refugia (Wallis and Trewick 2009; Marske and Boyer 2022). These regions were thought to have consisted of podocarp-broadleaf and beech forests that otherwise could not have survived in the colder and more arid climates brought on by glaciation (Buckley et al. 2015; Marske and Boyer 2022).

Other causes, such as Pleistocene/Holocene volcanic activity, also have been suggested (Barrell et al. 2005). Some studies have identified lineage divergence in animal species that are Pliocene in origin or predate the Pleistocene (e.g., Chapple et al. 2009; Marske et al. 2011; Withers et al. 2021), while the same pattern in other groups are speculated to have originated later (e.g., Painting et al. 2017). Ultimately, such trends in diversity in the North Island could be due to a combination of influences, having been first formed due to the Pliocene sea strait and then maintained or reinforced through Pleistocene glacial cycling, where many species would have experienced large contractions in their natural ranges (Buckley et al. 2015).

The degree to which historical events would have an effect on a species is dependent on the evolutionary history of that species. The ancestor of *Lophomyrtus* is believed to have been present in New Zealand since at least the early Miocene, potentially colonising the newly emergent landmass after Oligocene drowning (Vasconcelos et al. 2017;

Table 4 Characteristics of the 12 microsatellite markers developed for *Lophomyrtus bullata* and used in this study, including the repeat motif identified in the original sequence, the allele size range detected in screened samples, and the fluorescent dye used with each marker during genotyping

Locus	Primer Sequence (5'−3')	Repeat motif	Allele size range (bp)	M13 dye used
LB55	F: TCTACGGCCAAGGAAAGTGC R: TGCCAGTTGATTAAGGGTAAC	AT	274–296	FAM
LB44	F: CGCCCTTTGATTGATCGCTG R: TGCCACGTGTAAGGTTGGAC	TC	313–335	VIC
LB54	F: TCGTCACACTTTATGGCCGC R: GAAGCCTTTCCCCCTCGATC	GA	244–256	NED
LB42	F: ATCACTGATAAGGCCACCGG R: ACAGACAATCCCCGGAAGAC	TG	262–272	FAM
LB25	F: GACCAAGTCATCGGTCTTC R: TCAGATTCCAACAGGACAAG	AG	285–290	VIC
LB31	F: AGGGTATCAGGTTTTGGGCC R: TGTTCAGATGGTAGTCTCGC	CT	353–365	NED
LB41	F: TTTTTGACTGGGGTGGGTCC R: TAGTGGGTGTGATGGATGGC	CA	219–229	FAM
LB33	F: GCTCAGGCCAGGAAAATCATG R: GACACAGGGCTTGCATTTGG	CT	265–291	VIC
LB30	F: CATGCACAAGTACATCCCCG R: TTACATATCCTGGCGGCTGC	GA	339–346	NED
LB47	F: CATTTGCCTGCCTCATCCAC R: TTCATGGGTCACAGAAGCCC	CT	194–205	FAM
LB40	F: CATGACGCCTTGTGTTCTGTG R: AATTCCAGTTACCCTCGCCG	CT	280–290	VIC
LB39	F: GCTTTGTGTTCTTCCTTGGCC R: ACGGTGAGGTGGTTTTGGG	CA	287–303	NED

Smissen et al. 2022). *L. bullata* and *L. obcordata* are believed to have diverged ~ 3.6–5.6 mya (Thornhill et al. 2015). Therefore, it is possible that both Pliocene marine retreat and Pleistocene glaciation had an effect on the distribution of the species. Marine or glacial retreat are also possible explanations for the north-south split observed in *L. bullata*. The intersection of the two genotype groups occurs near the 38°S latitude, which fits with the explanation of recolonisation after marine or glacial retreat and suggests that current populations north of 38° may represent either refugia or were more quickly recolonised. Recolonisation south of 38° may have resulted in reduced genetic diversity within populations due to founder effects and lower genetic differentiation among populations as a consequence of more recent shared ancestry.

The north-south pattern observed in *L. bullata* is also evident in the genetic diversity of populations. Results from regression analyses indicate a strong relationship between decreasing He, Na, and latitude, with the Northland (SF, MB) and Coromandel (CFP) sites exhibiting higher genetic diversity (He) than other sites. In addition, the Northland (SF) and Auckland (HNA) locations have the highest number of private alleles, though this is still relatively low at just three (Table 4). Extant populations in regions of suspected former glacial refugia are sometimes observed to be more genetically diverse or distinct compared to other regions (Hewitt 2000; Byrne 2008), again supporting these upper North Island populations as potential past sites of glacial refugia for *L. bullata*. Distinct patterns of interspecific diversity in the upper North Island of New Zealand have been observed in both plant (McGlone 1985; Gardner et al. 2004) and invertebrate species (Buckley et al. 2015; Ellis et al. 2015; Trewick et al. 2022). If these upper North Island regions were the sole source for recolonisation after glacial retreat, this could have led to a serial expansion of *L. bullata* populations further south, with each new wave of colonisations being pioneered by an ever-diminishing gene pool and the genetic diversity of the newly colonised populations being further impacted by founder effects (Barton and Charlesworth 1984), resulting in comparatively lower genetic diversity within each newly established population. Such a scenario could potentially explain the north to south gradient observed in populations of *L. bullata* today, and could also have contributed to the overall low genetic diversity observed within the species.

However, it is unclear if the upper North Island region was the sole site of refugia for *L. bullata* during the LGM. *L. bullata* is generally found in lowland forests and would have been heavily restricted in its range during this period, but it is possible that populations survived in microrefugia in other regions during the Pleistocene, and that these regions also contributed to recolonisation. The higher levels of diversity found in the Northland, Coromandel, and Auckland could instead be a reflection of these refugia being the most extensive and stable in terms of forest coverage during this period or from the convergence of individuals from

other refugia. Survival of species in multiple microrefugia throughout the Pleistocene has been suggested for other New Zealand species (e.g., Buckley et al. 2010), as well as for species in other parts of the world (Nevill et al. 2010; Shi et al. 2014).

The north to south genetic cline could have additionally resulted from or have been contributed to by volcanic activity in the central North Island. Due to the location of the populations comprising the gradient, this volcanic activity would most likely have been originated from the Taupō volcanic zone. The Taupō volcanic zone has been the site of repeated wide-scale eruptions for the last 2 Ma (Wilmshurst and McGlone 1996; Barker et al. 2021). Vegetation in the immediate surroundings of the eruptions would have been severely impacted by basaltic lava flows and tephra (Briggs and McDonough 1990; Goles et al. 1996; Wilmshurst and McGlone 1996). Such events would have eliminated much of *L. bullata* and acted as a barrier to gene flow between surviving populations. Limited gene flow would have increased divergence between sites in the north and south, and the gradient pattern observed in the Waikato populations (TOT, MAN, OM) could be the result of secondary contact. Therefore, it is possible that the north-south pattern observed in *L. bullata* was first established by recolonisation after marine retreat and strengthened by the combined effects of glacial cycling and volcanic activity.

Genetic structure of *Lophomyrtus bullata*: regional genetic structure

Beyond the north-south pattern, populations of *L. bullata* further resolved into groups with regional patterns most clearly represented in the $K=5$ STRUCTURE results (Figs. 3 and 4). At this level, the five genetic groups roughly correspond to the north, the Waikato, East Cape, Taranaki/Whanganui, and the south. The majority of the regional groups identified by STRUCTURE contain a high degree of admixture. This admixture is most evident between populations on the borders of each group, which suggests current or recent gene flow among neighbouring regions. The significant result of the Mantel tests (Table 2) suggests that distance may be a factor in limiting gene flow between locations, potentially driving the genetic differences between some of the regional groups. Gene flow in *L. bullata* is largely through bird dispersed seeds and bee-mediated pollination (de Lange 2022). The latter has the potential to be more restricted by range compared to other mechanisms (e.g., wind pollination), which could result in more restricted gene flow (Wessinger 2021). Populations that are closer in proximity would be more likely to share pollinators, exchange genetic material, and therefore be more genetically similar. More distantly distributed populations would be less likely to exchange genetic material, accumulate genetic differences, and become increasingly diverged (Rousset 1997). However, this effect could be counteracted by the dispersal of seeds by birds, which would result in reduced population structure (e.g., Jesson 2007), potentially explaining the high degree of admixture and the relatively low overall F_{ST} value.

Genetic structure of *Lophomyrtus bullata*: East Cape

Individuals from the two East Cape populations (ECO, ECT) consistently form a distinct genetic group across all analyses and have significantly higher average pairwise F_{ST} values compared to all other *L. bullata* populations. A similar genetic distinction for East Cape populations has been noted across many other species, including stick insects (Buckley et al. 2010), beetles (Marske et al. 2009), cicadas (Ellis et al. 2015), and frogs (Fouquette Jr 1975). Several hypotheses have been suggested to explain the phenomenon, with the most supported suggesting that the northern East Cape acted as a glacial refugium during the Pleistocene (Marske et al. 2009; Buckley et al. 2010; Ellis et al. 2015). If this was the case for *L. bullata*, then it may explain why the East Cape populations are genetically distinct from those in other regions. Alternatively, Gardner et al. (2004) suggested that a similar pattern found in *Metrosideros* trees resulted from the isolation of the East Cape populations from the Coromandel due to volcanic activity along the Bay of Plenty coastline during the Holocene. The STRUCTURE results of *L. bullata* indicate some genetic similarity between the Tauranga (KM) population and the East Cape populations (Fig. 3), which could be the result of more recent gene flow between reconnected populations following isolation of the East Cape due to volcanic activity. Future work would benefit from the inclusion of samples from the Rotorua region, from which we were unable to obtain sampling permission.

Genetic structure of *Lophomyrtus bullata*: South Island

The results indicate a relationship between the Taranaki/Whanganui (MM, KR, HB, WHN) populations and the individuals from the one sampled South Island site (PBS). The relationship is reflected in the low pairwise F_{ST} values, the $K=4$ and $K=5$ STRUCTURE results, with the South Island population sharing a high proportion of membership with the Taranaki/Whanganui populations (Figs. 3 and 4), and the Neighbor-Net results. Similar geographic patterns have been observed in extinct Moa species (Bunce et al. 2009), species of skinks (Greaves et al. 2007, 2008; Liggins et al. 2008; O'Neill et al. 2008), geckos (Nielsen et al. 2011), as well as other plant species (Prebble et al. 2018). The pattern

has been suggested to be due to the presence of a land bridge between the north-western South Island and Cape Egmont during the Pleistocene due to lowered sea levels (Lewis et al. 1994; Trewick and Bland 2012). The land bridge was suggested to have been exposed intermittently between glacial cycles to varying degrees (Lloyd 2003), with the continental shelf between Cape Farewell and Cape Egmont the last region to be inundated by rising post-glacial sea levels (Lewis et al. 1994). The climate of the land bridge is modelled to have been cool and arid, like the majority of the lower North Island during this period, and therefore might have limited dispersal (Marske and Boyer 2022). However, others have suggested that the land bridge was forested, at least at certain times between glacial cycles (Marra et al. 2009).

One possible explanation for this relationship between the Taranaki and South Island populations is that *L. bullata* was present in glacial refugia in the northern South Island (Heenan and Mitchell 2003; McGlone et al. 2010). This may suggest that the populations were reconnected by the Pleistocene North-South Island land bridge. If this were the case, we might expect to see more of a distinction between the South Island population and the lower North Island, considering, like the northern North Island, the northern South Island is a region observed to have greater endemism (Wardle 1963; McGlone 1985). However, this is not observed in the analyses, with the DAPC, and to some extent, NeighborNet results suggesting that the South Island population and the lower North Island populations are all genetically quite similar. However, it should be noted that this result could be impacted by the limited number of South Island populations included in the analysis. Further sampling across the South Island distribution of *L. bullata* should be a priority for future work.

Hybridization between *L. bullata* and *L. obcordata*

In analyses that included all samples, the patterns revealed indicate that, despite hybridisation between *L. bullata* and *L. obcordata*, based on the limited sampling here, at most locations, the two species remain genetically distinct. However, it was surprising that the initial STRUCTURE results did not separate species first, though this could be due to the reliance of STRUCTURE on the HWE criterion (Pritchard et al. 2000), which can be affected by sample sizes. As the lower North Island sites may form a 'good' HWE population due to their high genetic similarity, at $K=2$, this would place all remaining populations into the other genetic group; the low ΔK value for $K=2$ could be considered potential evidence of this (Fig. 7). In STRUCTURE results at $K>2$, and all other analyses, *L. obcordata* and *L. bullata* are well distinguished and the two locations with morphologically intermediate individuals (WA and MOW) were found to be genetically intermediate. Although it was not feasible in the current study, interpretations of past and contemporary hybridization in *Lophomyrtus* would benefit from broader sampling of *L. obcordata*.

Interspecific hybridization can introduce substantial genetic variation, potentially exceeding that generated by mutation if hybridization events are frequent (Anderson 1949; Grant and Grant 1994; Arnold and Martin 2009; Abbott et al. 2013; Dittrich-Reed and Fitzpatrick 2013). Consistent with this, the putative *Lophomyrtus* hybrid populations exhibited higher expected heterozygosity than most *L. bullata* and *L. obcordata* sites, indicating elevated genetic diversity (Table 1). Such an increase in diversity resulting from hybridisation could result in increased adaptive potential (e.g., Rattenbury 1962; Ogden 1989; Becker et al. 2013). Evolutionary advantages gained through hybridization have been suggested for several other New Zealand plant groups, including *Pachycladon* (Becker et al. 2013), *Nothofagus* (Ogden 1989), *Sophora* (Shepherd and Heenan 2021), and *Coprosma* (Rattenbury 1962). In certain environments, it has been suggested that putative *L. bullata* x *L. obcordata* hybrid individuals outcompete *L. bullata* individuals (de Lange 2022), possibly indicating greater fitness for hybrids in those environments.

Importantly, hybridisation between *L. bullata* and *L. obcordata* could provide new advantageous genetic combinations for myrtle rust resistance. Exchange and recombination of genetic material between individuals of *L. bullata* and *L. obcordata* could potentially allow for both species to acquire new trait combinations for pathogen resistance. Interestingly, it has been observed that while populations of *L. bullata* are declining rapidly, populations of *L. obcordata*, though also declining, are doing so at a slower rate (Prasad et al. 2022). Though there could be other causes for this slower decline, it is possible that *L. obcordata* harbours greater resistance against myrtle rust that is not present in *L. bullata* but that could be shared through hybridisation. A similar scenario has been described in New Zealand alpine species of *Pachycladon*, where past introgression from hybridisation led to the transfer of chemotypes of glucosinolate hydrolysis, which are used in herbivore and pathogen defence (Voelckel et al. 2008; Becker et al. 2013). Additionally, the hybridisation of *L. bullata* and *L. obcordata* could lead to gene stacking for resistance, a strategy commonly used in plant breeding programs (Rogozina et al. 2021). Both are possible scenarios where hybridisation with *L. obcordata* could potentially benefit the future preservation of *L. bullata*; however, no data currently exist for potential outbreeding depression in interspecific hybrids of *Lophomyrtus*, which could pose a significant disadvantage to novel genetic combinations.

Implications for conservation

Although the full impact of myrtle rust is not yet realised, its arrival in New Zealand has had a dramatic impact on the endemic myrtle species *Lophomyrtus bullata* (Smith et al. 2020; Sutherland et al. 2020; Toome-Heller et al. 2020). The fast-spreading pathogen has already infected multiple populations of *L. bullata* from Northland to the Marlborough sounds, resulting in widespread dieback and mortality in trees of all ages (Beyond-Myrtle-Rust 2020). Infection by myrtle rust and the corresponding effects on plant fecundity could act as an extreme selective pressure for *L. bullata*. The relatively low genetic diversity observed within populations of *L. bullata* suggests that the species is potentially limited in its ability to adapt to such a pressure (Kardos et al. 2021). Tree mortality could cause population numbers to decline rapidly, increasing the effects of genetic drift, decreasing genetic variation and increasing inbreeding, potentially resulting in even further reduced fitness for the species.

The population structure of *L. bullata* shows that while there are low to moderate levels of genetic differentiation present between populations, there is distinct regional genetic variation. Therefore, care should be taken that individuals from the appropriate regional locations are used for sourcing regeneration plantings. Regarding seed banking, priority might be given to Northland, Coromandel, and Auckland populations as they contain a higher degree of intraspecific genetic diversity (e.g., higher expected heterozygosity, and, though few were detected, a higher number of private alleles (Table 1)), but it should also be ensured that all regional genetic groups are effectively represented in germplasm collections. It would also seem prudent to protect, monitor, and potentially seed bank *L. bullata* x *L. obcordata* hybrid populations as they could possess unique genetic combinations that may be important to conserving the *Lophomyrtus* lineage.

The results presented here also could be used as a baseline to measure the ongoing effects of myrtle rust on the diversity and population dynamics of *L. bullata*. A comparable study conducted in the future would allow changes in genetic diversity to be tracked and quantified. This would be particularly relevant for populations where myrtle rust has only recently been established (e.g., MAN) and populations that currently have no observations of myrtle rust (e.g., OM). Additionally, the inclusion of sites where seeds have been collected in the past (e.g., ECT) would allow for comparisons between past and current levels of genetic diversity. A similar approach was used to investigate *Banksia brownii*, a woody shrub species endemic to Australia that has undergone a considerable reduction in numbers and localised extinction due to the plant pathogen *Phytophthora cinnamomi* (Coates et al. 2015). That study used comparisons between extant populations and seed collections from extinct populations to estimate the loss of genetic diversity following population extinction. It was observed that the species lost over a third of the total genetic diversity and 22 out of 88 private alleles due to the effects of *P. cinnamomi* (Coates et al. 2015). A similar approach could provide important insights into how *L. bullata* is responding to myrtle rust over time.

Conclusions

This study provides a detailed first estimate of the genetic variation within the threatened species *Lophomyrtus bullata*. The species has relatively low levels of genetic diversity compared to other species with similar life histories. How this variation is partitioned across the geographic distribution of the species suggests it may at one time have been affected by a considerable reduction in range, possibly during the Last Glacial Maximum, which may have resulted in populations being restricted to the upper North Island, where the greatest genetic variation is currently found. Subsequent range expansion from the upper North Island to the south could have led to recolonised populations harbouring lower genetic diversity compared to the source populations due to founder effects. Such patterns could also have been influenced by volcanic activity isolating populations in the north and south and increasing genetic differentiation. Significant regional genetic patterns observed are likely attributed to the effects of past climatic events, isolation by distance, dispersal mechanisms of the species, multiple glacial refugia, or a combination of all of these factors. *L. bullata* is threatened with extreme range reduction in the near future due to the effects of the pathogenic fungus myrtle rust, which is causing rapid population decline, which will further decrease genetic diversity, increasing the species' risk of extinction further. The results presented here provide critical information to support the conservation and potential restoration of *Lophomyrtus bullata*, enhancing the effectiveness of seed banking, population preservation, and regeneration initiatives.

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Author contributions The project conception was by V.S, study design was performed by V.S. and A.M., field work and data collection were performed by A.M., and both authors contributed to data analysis. The first draft of the manuscript was prepared by A.M. and subsequent

drafts were revised by both authors. Both authors have reviewed and approve of the final manuscript.

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Data availability The microsatellite datasets generated during this study are available at the Dryad repository (https://doi.org/10.5061/dryad.vdncjsz96).

Declarations

Competing interests The authors declare no competing interests.

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References

Abbott R, Albach D, Ansell S, Arntzen JW, Baird SJ, Bierne N, Boughman J, Brelsford A, Buerkle CA, Buggs R (2013) Hybridization and speciation. J Evol Biol 26:229–246

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Mol Ecol 17:5177–5188

Allan HH (1929) Illustrations of wild hybrids in the New Zealand flora. VI. Genetica, p 11

Allan HH (1961) Flora of New Zealand, vol 1. Government Printer, Wellington

Anderson E (1949) Introgressive hybridization. Biol Rev 28:280–307

Arnold ML, Martin NH (2009) Adaptation by introgression. J Biol 8:1–3

Balvanera P, Pfisterer AB, Buchmann N, He JS, Nakashizuka T, Raffaelli D, Schmid B (2006) Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol Lett 9:1146–1156

Barker SJ, Wilson CJ, Illsley-Kemp F, Leonard GS, Mestel ER, Mauriohooho K, Charlier BL (2021) Taupō: an overview of New Zealand's youngest supervolcano. N Z J Geol Geophys 64:320–346

Barnaud A, Houliston GJ (2010) Population genetics of the threatened tree daisy *Olearia gardneri* (Asteraceae), conservation of a critically endangered species. Conserv Genet 11:1515–1522

Barrell DJA, Alloway B, Shulmeister J, Newnham R (2005) Towards a climate event stratigraphy for New Zealand over the past 30,000 years. Institute of Geological and Nuclear Sciences Science Report 7:12

Barton NH, Charlesworth B (1984) Genetic revolutions, founder effects, and speciation. Annu Rev Ecol Syst 15:133–164

Becker M, Gruenheit N, Steel M, Voelckel C, Deusch O, Heenan PB, McLenachan PA, Kardailsky O, Leigh JW, Lockhart PJ (2013) Hybridization may facilitate in situ survival of endemic species through periods of climate change. Nat Clim Chang 3:1039–1043

Beggs J (2001) The ecological consequences of social wasps (*Vespula* spp.) invading an ecosystem that has an abundant carbohydrate resource. Biol Conserv 99:17–28

Beresford RM, Turner R, Tait A, Paul V, Macara G, Zhidong DY, Lima L, Martin R (2018) Predicting the Climatic risk of Myrtle rust during its first year in New Zealand. New Z Plant Prot 71:332–347

Beresford RM, Shuey LS, Pegg GS (2020) Symptom development and latent period of *Austropuccinia psidii* (myrtle rust) in relation to host species, temperature, and ontogenic resistance. Plant Pathol 69:484–494

Best E (1925a) Games and pastimes of the Maori. A. R. Shearer, Wellington

Best E (1925b) The Maori canoe. A. R. Shearer, Wellington

Best E (1942) Forest Lore of the Maori. E. C. Keating, Wellington

Beyond-Myrtle-Rust (2020) Myrtle rust update: mature native trees now dying. Manaaki Whenua Landcare Research. https://www.landcareresearch.co.nz/news/myrtle-rust-update-mature-native-trees-now-dying

Blackburn TM, Bellard C, Ricciardi A (2019) Alien versus native species as drivers of recent extinctions. Front Ecol Environ 17:203–207

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques 31:25–28

Bradshaw R, Bellgard S, Black A, Burns B, Gerth M, McDougal R, Scott P, Waipara N, Weir B, Williams N (2020) *Phytophthora agathidicida*: research progress, cultural perspectives and knowledge gaps in the control and management of Kauri dieback in New Zealand. Plant Pathol 69:3–16

Brasier C, Buck K (2001) Rapid evolutionary changes in a globally invading fungal pathogen (Dutch elm disease). Biol Invasions 3:223–233

Briggs R, McDonough W (1990) Contemporaneous convergent margin and intraplate magmatism, North Island, New Zealand. J Petrol 31:813–851

Broadhurst LM, Young AG, Murray BG (2008) AFLPs reveal an absence of geographical genetic structure among remnant populations of Pohutukawa (*Metrosideros excelsa*, Myrtaceae). N Z J Bot 46:13–21

Brownstein MJ, Carpten JD, Smith JR (1996) Modulation of non-templated nucleotide addition by *Taq* DNA polymerase: primer modifications that facilitate genotyping. Biotechniques 20:1004–1010

Buckley TR, Marske KA, Attanayake D (2009) Identifying glacial refugia in a geographic parthenogen using palaeoclimate modelling and phylogeography: the New Zealand stick insect *Argosarchus horridus* (White). Mol Ecol 18:4650–4663

Buckley TR, Marske K, Attanayake D (2010) Phylogeography and ecological niche modelling of the New Zealand stick insect *Clitarchus hookeri* (White) support survival in multiple coastal refugia. J Biogeogr 37:682–695

Buckley TR, Krosch M, Leschen RA (2015) Evolution of New Zealand insects: summary and prospectus for future research. Austral Entomol 54:1–27

Bulgarella M, Trewick SA, Minards NA, Jacobson MJ, Morgan-Richards M (2014) Shifting ranges of two tree weta species (*H. emideina* spp.): competitive exclusion and changing climate. J Biogeogr 41:524–535

Bunce M, Worthy TH, Phillips MJ, Holdaway RN, Willerslev E, Haile J, Shapiro B, Scofield RP, Drummond A, Kamp PJ (2009) The evolutionary history of the extinct ratite moa and New

Zealand Neogene paleogeography. Proc Natl Acad Sci U S A 106:20646–20651
Byrne M (2008) Evidence for multiple refugia at different time scales during Pleistocene climatic oscillations in Southern Australia inferred from phylogeography. Quat Sci Rev 27:2576–2585
Cadotte MW, Cardinale BJ, Oakley TH (2008) Evolutionary history and the effect of biodiversity on plant productivity. Proc Natl Acad Sci USA 105:17012–17017
Cambie RC, Ferguson LR (2003) Potential functional foods in the traditional Maori diet. Mutat Research/Fundamental Mol Mech Mutagen 523–524:109–117
Carnegie AJ, Kathuria A, Pegg GS, Entwistle P, Nagel M, Giblin FR (2016) Impact of the invasive rust *Puccinia psidii* (myrtle rust) on native myrtaceae in natural ecosystems in Australia. Biol Invasions 18:127–144
Caye K, Deist TM, Martins H, Michel O, François O (2016) TESS3: fast inference of spatial population structure and genome scans for selection. Mol Ecol Resour 16:540–548
Ceballos G, Ehrlich PR, Dirzo R (2017) Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. Proc Natl Acad Sci U S A 114:E6089–E6096
Chapple DG, Ritchie PA, Daugherty CH (2009) Origin, diversification, and systematics of the New Zealand skink fauna (Reptilia: Scincidae). Mol Phylogenet Evol 52:470–487
Charlesworth D, Willis JH (2009) The genetics of inbreeding depression. Nat Rev Genet 10:783–796
Cheeseman TF (1906) Manual of the New Zealand flora. Government Printer
Clearwater MJ, Noe ST, Manley-Harris M, Truman GL, Gardyne S, Murray J, Obeng-Darko SA, Richardson SJ (2021) Nectary photosynthesis contributes to the production of mānuka (*Leptospermum scoparium*) floral nectar. New Phytol 232:1703–1717
Coates DJ, McArthur SL, Byrne M (2015) Significant genetic diversity loss following pathogen driven population extinction in the rare endemic *Banksia brownii* (Proteaceae). Biol Conserv 192:353–360
Cockayne L (1918) Notes on New Zealand floristic botany, including descriptions of new species, etc. (3). Trans Proc R Soc N Z 50:161–191
Community TG (2024) The galaxy platform for accessible, reproducible, and collaborative data analyses: 2024 update. Nucleic Acids Res 52:W83–W94
Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014
Craig J, Anderson S, Clout M, Creese B, Mitchell N, Ogden J, Roberts M, Ussher G (2000) Conservation issues in New Zealand. Annu Rev Ecol Syst 31:61–78
Cullingham CI, Miller JM, Peery RM, Dupuis JR, Malenfant RM, Gorrell JC, Janes JK (2020) Confidently identifying the correct K value using the ΔK method: when does K = 2? Mol Ecol 29:862–869
Daily GC (1997) Nature's services: societal dependence on natural ecosystems. Island
de Lange PJ (2014) A revision of the New Zealand *Kunzea ericoides* (Myrtaceae) complex. PhytoKeys. https://doi.org/10.3897/phytokeys.40.7973
de Lange PJ (2022) *Lophomyrtus bullata* Fact Sheet. https://www.nzpcn.org.nz/flora/species/lophomyrtus-bullata/
de Lange PJ, Rolfe JR, Courtney SP, Champion PD, Perrie LR, Beadel SM, Ford KA, Breitwiser I, Schonberger I, Hindsmarsh-Walls R, Heenan PB, Ladley K (2018) Conservation status of New Zealand Indigenous vascular plants, 2017. New Zealand threat classification series. Department of Conservation, Wellington
DeWoody JA, Harder AM, Mathur S, Willoughby JR (2021) The long-standing significance of genetic diversity in conservation. Mol Ecol 30:4147–4154
Dharmarajan G, Beatty WS, Rhodes OE Jr (2013) Heterozygote deficiencies caused by a Wahlund effect: dispelling unfounded expectations. J Wildl Manag 77:226–234
Dittrich-Reed DR, Fitzpatrick BM (2013) Transgressive hybrids as hopeful monsters. Evol Biol 40:310–315
Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15
Du Plessis E, Granados GM, Barnes I, Ho W, Alexander B, Roux J, McTaggart AR (2019) The pandemic strain of *Austropuccinia psidii* causes myrtle rust in New Zealand and Singapore. Australas Plant Pathol 48:253–256
Earl DA, VonHoldt BM (2012) Structure harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361
Ellis EA, Marshall DC, Hill KB, Owen CL, Kamp JP, Simon C (2015) Phylogeography of six codistributed New Zealand cicadas and their relationship to multiple biogeographical boundaries suggest a re-evaluation of the Taupo line. J Biogeogr 42:1761–1775
Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. Annu Rev Ecol Syst 24:217–242
Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620
Fernandez-Winzer L, Berthon KA, Entwistle P, Manea A, Winzer N, Pegg GS, Carnegie AJ, Leishman MR (2020) Direct and indirect community effects of the invasive plant pathogen *Austropuccinia psidii* (myrtle rust) in eastern Australian rainforests. Biol Invasions 22:2357–2369
Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ (2012) Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186–194
Fisher MC, Gurr SJ, Cuomo CA, Blehert DS, Jin H, Stukenbrock EH, Stajich JE, Kahmann R, Boone C, Denning DW (2020) Threats posed by the fungal kingdom to humans, wildlife, and agriculture. MBio 11:e00449–e00420
Fleming CA (1979) The geological history of New Zealand and its biota. Auckland University, Auckland, New Zealand
Fouquette M Jr (1975) Speciation in chorus frogs. I. Reproductive character displacement in the *Pseudacris nigrita* complex. Syst Biol 24:16–23
Frankel O, Soule ME (1981) Conservation and evolution. Cambridge University Press, Cambridge
Fungtammasan A, Ananda G, Hile SE, Su MS, Sun C, Harris R, Medvedev P, Eckert K, Makova KD (2015) Accurate typing of short tandem repeats from genome-wide sequencing data and its applications. Genome Res 25:736–749
Gardner RC, De Lange PJ, Keeling DJ, Bowala T, Brown HA, Wright SD (2004) A late quaternary phylogeography for *Metrosideros* (Myrtaceae) in New Zealand inferred from Chloroplast DNA haplotypes. Biol J Linn Soc 83:399–412
Gemmell MR, Shepherd LD, Zuccarello GC, Perrie LR (2022) Phylogeography of the widespread New Zealand tree lancewood/horoeka (*Pseudopanax crassifolius*; Araliaceae). N Z J Bot : 1–17
Glen M, Alfenas A, Zauza E, Wingfield M, Mohammed C (2007) *Puccinia psidii*: a threat to the Australian environment and economy—a review. Australas Plant Pathol 36:1–16
Goles G, Briggs R, Rosenberg M (1996) Late pliocene stratigraphic succession and volcanic evolution of Karioi volcano, Western North Island, New Zealand. N Z J Geol Geophys 39:283–294
Grant PR, Grant BR (1994) Phenotypic and genetic effects of hybridization in Darwin's finches. Evolution 48:297–316

Greaves SN, Chapple DG, Gleeson DM, Daugherty CH, Ritchie PA (2007) Phylogeography of the spotted skink (*Oligosoma lineoocellatum*) and green skink (*O. chloronoton*) species complex (Lacertilia: Scincidae) in New Zealand reveals pre-Pleistocene divergence. Mol Phylogenet Evol 45:729–739

Greaves SN, Chapple DG, Daugherty CH, Gleeson DM, Ritchie PA (2008) Genetic divergences pre-date Pleistocene glacial cycles in the New Zealand speckled skink, *Oligosoma infrapunctatum*. J Biogeogr 35:853–864

Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species. Philos Trans R Soc Lond B Biol Sci 351:1291–1298

Hedrick PW, Kalinowski ST (2000) Inbreeding depression in conservation biology. Annu Rev Ecol Syst. https://doi.org/10.1146/annurev.ecolsys.31.1.139

Heenan P, Mitchell A (2003) Phylogeny, biogeography and adaptive radiation of *Pachycladon* (Brassicaceae) in the mountains of South Island, New Zealand. J Biogeogr 30:1737–1749

Heenan PB, Millar TR, Smissen RD, McGlone MS, Wilton AD (2017) Phylogenetic measures of neo- and palaeo-endemism in the Indigenous vascular flora of the New Zealand archipelago. Aust Syst Bot 30:124–133

Heenan PB, McGlone MS, Mitchell CM, Cheeseman DF, Houliston GJ (2022) Genetic variation reveals broad-scale biogeographic patterns and challenges species' classification in the *Kunzea ericoides* (kānuka; Myrtaceae) complex from New Zealand. N Z J Bot 60:2–26

Hewitt G (2000) The genetic legacy of the quaternary ice ages. Nature 405:907–913

Hindrikson M, Remm J, Pilot M, Godinho R, Stronen AV, Baltrūnaité L, Czarnomska SD, Leonard JA, Randi E, Nowak C (2017) Wolf population genetics in Europe: a systematic review, meta-analysis and suggestions for conservation and management. Biol Rev 92:1601–1629

Hiroa TR (1949) The coming of the Maori. Māori Purposes Fund Board, Wellington, NZ

Ho WH, Baskarathevan J, Griffin RL, Quinn BD, Alexander BJR, Havell D, Ward NA, Pathan AK (2019) First report of Myrtle rust caused by on Metrosideros kermadecensis on Raoul Island and on M. excelsa in Kerikeri, New Zealand. Plant Dis 103:2128–2128

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Mol Biol Evol 23:254–267

Jesson LK (2007) Ecological correlates of diversification in New Zealand angiosperm lineages. N Z J Bot 45:35–51

Jo I, Bellingham PJ, McCarthy JK, Easdale TA, Padamsee M, Wiser SK, Richardson SJ (2022) Ecological importance of the Myrtaceae in New Zealand's natural forests. J Veg Sci 33:e13106

Jombart T (2008) Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:1–15

Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281

Kardos M, Armstrong EE, Fitzpatrick SW, Hauser S, Hedrick PW, Miller JM, Tallmon DA, Funk WC (2021) The crucial role of genome-wide genetic variation in conservation. Proc Natl Acad Sci U S A 118:e2104642118

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649

Kelly D, Ladley JJ, Robertson A, Anderson SH (2010) Mutualisms with the wreckage of an avifauna: the status of bird pollination and fruit-dispersal in New Zealand. N Z J Ecol 34:66–85

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Mol Ecol Resour 15:1179–1191

Kueffer C (2017) Plant invasions in the anthropocene. Science 358:724–725

Lewis KB, Carter L, Davey FJ (1994) The opening of Cook Strait: interglacial tidal scour and aligning basins at a subduction to transform plate edge. Mar Geol 116:293–312

Liggins L, Chapple DG, Daugherty CH, Ritchie PA (2008) A sine of restricted gene flow across the alpine fault: phylogeography of the New Zealand common skink (*Oligosoma Nigriplantare polychroma*). Mol Ecol 17:3668–3683

Lloyd BD (2003) The demographic history of the New Zealand short-tailed bat *Mystacina tuberculata* inferred from modified control region sequences. Mol Ecol 12:1895–1911

Lockhart PJ, McLenachan PA, Havell D, Glenny D, Huson D, Jensen U (2001) Phylogeny, radiation, and transoceanic dispersal of New Zealand alpine buttercups: molecular evidence under split decomposition. Ann Mo Bot Gard. https://doi.org/10.2307/3298586

Maki M (2003) Population genetics of threatened wild plants in Japan. J Plant Res 116:169–174

Marra M, Crozier M, Goff J (2009) Palaeoenvironment and biogeography of a late MIS 3 fossil beetle fauna from South Taranaki, New Zealand. J Quat Sci 24:97–107

Marske KA, Boyer SL (2022) Phylogeography reveals the complex impact of the last glacial maximum on New Zealand's terrestrial biota. J R Soc N Z (1):22. https://doi.org/10.1080/03036758.2022.2079682

Marske KA, Leschen RA, Barker GM, Buckley TR (2009) Phylogeography and ecological niche modelling implicate coastal refugia and trans-alpine dispersal of a New Zealand fungus beetle. Mol Ecol 18:5126–5142

Marske KA, Leschen RA, Buckley TR (2011) Reconciling phylogeography and ecological niche models for New Zealand beetles: looking beyond glacial refugia. Mol Phylogenet Evol 59:89–102

Melesse KA (2017) Molecular phylogeny of the genus *Metrosideros* and population genetics of some New Zealand species within the genus. Doctoral dissertation, University of Canterbury

McGlone M (1985) Plant biogeography and the late Cenozoic history of New Zealand. N Z J Bot 23:723–749

McGlone M, Duncan R, Heenan P (2001) Endemism, species selection and the origin and distribution of the vascular plant flora of New Zealand. J Biogeogr 28:199–216

McGlone MS, Newnham RM, Moar NT (2010) The vegetation cover of New Zealand during the last glacial maximum: do pollen records under-represent woody vegetation. Terra Australis 32:49–68

McLay TG, Tate JA, Gemmill CE, Holzapfel AS, Symonds VV (2022) Inferring the biogeography of New Zealand's only endemic holoparasitic plant, the threatened *Dactylanthus taylorii* (Mystropetalaceae). N Z J Bot 1–23

Meudt HM, Prebble JM, Lehnebach CA (2015) Native New Zealand forget-me-nots (*Myosotis*, Boraginaceae) comprise a Pleistocene species radiation with very low genetic divergence. Plant Syst Evol 301(5):1455–1471

Millar TR, Heenan PB, Wilton AD, Smissen RD, Breitwieser I (2017) Spatial distribution of species, genus and phylogenetic endemism in the vascular flora of New Zealand, and implications for conservation. Aust Syst Bot 30:134–147

Nevill PG, Bossinger G, Ades PK (2010) Phylogeography of the world's tallest angiosperm, *Eucalyptus regnans*: evidence for multiple isolated quaternary refugia. J Biogeogr 37:179–192

Nevill PG, Bradbury D, Williams A, Tomlinson S, Krauss SL (2014) Genetic and palaeo-climatic evidence for widespread persistence of the coastal tree species *Eucalyptus gomphocephala* (Myrtaceae) during the Last Glacial Maximum. Ann Bot 113: 55–67

Newnham R, McGlone M, Moar N, Wilmshurst J, Vandergoes M (2013) The vegetation cover of New Zealand at the last glacial maximum. Quat Sci Rev 74:202–214

Nielsen SV, Bauer AM, Jackman TR, Hitchmough RA, Daugherty CH (2011) New Zealand geckos (Diplodactylidae): cryptic diversity in a post-Gondwanan lineage with trans-Tasman affinities. Mol Phylogenet Evol 59:1–22

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13:1143–1155

O'Neill SB, Chapple DG, Daugherty CH, Ritchie PA (2008) Phylogeography of two New Zealand lizards: Mccann's skink (*Oligosoma maccanni*) and the brown skink (*O. zelandicum*). Mol Phylogenet Evol 48:1168–1177

Ogden J (1989) On the coenospecies concept and tree migrations during the oscillations of the Pleistocene climate. J R Soc N Z 19:249–262

Painting CJ, Myers S, Holwell GI, Buckley TR (2017) Phylogeography of the New Zealand giraffe weevil *Lasiorhynchus barbicornis* (Coleoptera: Brentidae): a comparison of biogeographic boundaries. Biol J Linn Soc 122:13–28

Pathan AK, Cuddy W, Kimberly MO, Adusei-Fosu K, Rolando CA, Park RF (2020) Efficacy of fungicides applied for protectant and curative activity against myrtle rust. Plant Dis 104:2123–2129

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. Bioinformatics 28:2537–2539

Pegg G, Taylor T, Entwistle P, Guymer G, Giblin F, Carnegie A (2017) Impact of *Austropuccinia psidii* (myrtle rust) on Myrtaceae-rich wet sclerophyll forests in South East Queensland. PLoS One 12:e0188058

Prasad M, Schmid LM, Marshall A, Blanchon D, Renner MA, Baba Y, Padamsee M, de Lange P (2022) Ecological communities of Aotearoa/New Zealand species threatened by Myrtle rust (*Austropuccinia psidii* (G. Winter) Beenken): the flora and mycobiota of the endemic genus *Lophomyrtus* burret. Perspect Biosecur 7:34–70

Prebble JM, Meudt HM, Tate JA, Symonds VV (2018) Bolstering species delimitation in difficult species complexes by analyzing herbarium and common garden morphological data: a case study using the New Zealand native *Myosotis pygmaea* species group (Boraginaceae). Syst Bot 43:266–289

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Pyšek P, Hulme PE, Simberloff D, Bacher S, Blackburn TM, Carlton JT, Dawson W, Essl F, Foxcroft LC, Genovesi P (2020) Scientists' warning on invasive alien species. Biol Rev 95:1511–1534

Quijas S, Schmid B, Balvanera P (2010) Plant diversity enhances provision of ecosystem services: a new synthesis. Basic Appl Ecol 11:582–593

Rattenbury J (1962) Cyclic hybridization as a survival mechanism in the New Zealand forest flora. Evolution. https://doi.org/10.1111/j.1558-5646.1962.tb03227.x

Raymond M, Rousset F (1995) GENEPOP (web version 3.1 c): populations software for exact test and ecumenicism. J Hered 86:248–249

Ricciardi A (2007) Are modern biological invasions an unprecedented form of global change? Conserv Biol 21:329–336

Ridley G, Bain J, Bulman L, Dick M, Kay M (2000) Threats to New Zealand's Indigenous forests from exotic pathogens and pests. Sci Conserv 142:1–67

Rigling D, Prospero S (2018) *Cryphonectria parasitica*, the causal agent of chestnut blight: invasion history, population biology and disease control. Mol Plant Pathol 19:7–20

Riley M (1994) Māori healing and herbal: New Zealand ethnobotanical sourcebook. Viking Sevenseas

Rogozina EV, Beketova MP, Muratova OA, Kuznetsova MA, Khavkin EE (2021) Stacking resistance genes in multiparental interspecific potato hybrids to anticipate late blight outbreaks. Agronomy 11:115

Rossetto M, Slade R, Baverstock PR, Henry RJ, Lee LS (1999) Microsatellite variation and assessment of genetic structure in tea tree (*Melaleuca alternifolia*-Myrtaceae). Mol Ecol 8:633–643

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219–1228

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132:365–386

RStudio-Team (2020) RStudio: Integrated Development for RStudio. PBC, Boston, MA

Salmon JT (1980) The native trees of New Zealand. Craig Potton Publishing

Sampson J, Tapper S, Coates D, Hankinson M, Mcarthur S, Byrne M (2018) Persistence with episodic range expansion from the early Pleistocene: the distribution of genetic variation in the forest tree *Corymbia calophylla* (Myrtaceae) in south-western Australia. Biol J Linn Soc 123: 545–560

Sandhu K, Karaoglu H, Zhang P, Park R (2016) Simple sequence repeat markers support the presence of a single genotype of *Puccinia psidii* in Australia. Plant Pathol 65:1084–1094

Scheele BC, Pasmans F, Skerratt LF, Berger L, Martel A, Beukema W, Acevedo AA, Burrowes PA, Carvalho T, Catenazzi A (2019) Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science 363:1459–1463

Schoen DJ, Brown AHD (2001) The conservation of wild plant species in seed banks: attention to both taxonomic coverage and population biology will improve the role of seed banks as conservation tools. Bioscience 51:960–966

Shepherd LD, Heenan PB (2021) Phylogenomic analyses reveal a history of hybridisation and introgression between *Sophora* sect. Edwardsia (Fabaceae) species in New Zealand. N Z J Bot, 1–21

Shepherd LD, Perrie LR (2011) Microsatellite DNA analyses of a highly disjunct New Zealand tree reveal strong differentiation and imply a formerly more continuous distribution. Mol Ecol 20:1389–1400

Shi MM, Michalski SG, Welk E, Chen XY, Durka W (2014) Phylogeography of a widespread Asian subtropical tree: genetic east–west differentiation and climate envelope modelling suggest multiple glacial refugia. J Biogeogr 41:1710–1720

Smissen RD, de Lange PJ, Thorsen MJ, Ogle CC (2011) Species delimitation and genetic variation in the rare New Zealand endemic grass genus *Simplicia*. N Z J Bot 49:187–199

Smissen RD, Heenan PB, Maurin KJ (2022) New Zealand endemic *Neomyrtus* is sister to new Caledonian endemic *Myrtastrum* (Myrtaceae, Myrteae). N Z J Bot 60:184–197

Smith GR, Ganley BJ, Chagne D, Nadarajan J, Pathirana RN, Ryan J, Arnst EA, Sutherland R, Soewarto J, Houliston G, Marsh AT, Koot E, Carnegie AJ, Menzies T, Lee DJ, Shuey LS, Pegg GS (2020) Resistance of New Zealand Provenance *Leptospermum scoparium*, *Kunzea robusta*, *Kunzea linearis*, and *Metrosideros excelsa* to *Austropuccinia psidii*. Plant Disease 104:1771–1780

Stacy EA, Sakishima T, Tharp H, Snow N (2020) Isolation of *Metrosideros* ('Ohi'a) Taxa on O'ahu Increases with Elevation and Extreme Environments. J Hered 111: 103–118

Steane DA, Conod N, Jones RC, Vaillancourt RE, Potts BM (2006) A comparative analysis of population structure of a forest tree, *Eucalyptus globulus* (Myrtaceae), using microsatellite markers and quantitative traits. Tree Genetics & Genomes 2: 30–38

Stewart J, Ross-Davis A, Graça R, Alfenas A, Peever T, Hanna J, Uchida J, Hauff R, Kadooka C, Kim MS (2018) Genetic diversity of the Myrtle rust pathogen (*Austropuccinia psidii)* in the Americas and hawaii: global implications for invasive threat assessments. Forest Pathol 48:e12378

Suarez AV, Tsutsui ND (2008) The evolutionary consequences of biological invasions. Mol Ecol 17:351–360

Suggate RP (1978) The geology of New Zealand. Government Printer, Wellington

Sutherland R, Soewarto J, Beresford R, Ganley B (2020) Monitoring *Austropuccinia psidii* (myrtle rust) on New Zealand myrtaceae in native forest. New Z J Ecol 44

Symonds VV, Lloyd AM (2004) A simple and inexpensive method for producing fluorescently labelled size standard. Mol Ecol Notes 4:768–771

Szczecinska M, Sramko G, Wolosz K, Sawicki J (2016) Genetic diversity and population structure of the rare and endangered plant species *Pulsatilla patens* (L.) mill in East central Europe. Plos One p 11

Thornhill AH, Ho SY, Külheim C, Crisp MD (2015) Interpreting the modern distribution of Myrtaceae using a dated molecular phylogeny. Mol Phylogenet Evol 93:29–43

Toome-Heller M, Ho WWH, Ganley RJ, Elliott CEA, Quinn B, Pearson HG, Alexander BJR (2020) Chasing myrtle rust in New Zealand: host range and distribution over the first year after invasion (vol 71, pg 343, 2020). Australas Plant Pathol 49:327–328

Trewick S, Bland K (2012) Fire and slice: palaeogeography for biogeography at New Zealand's North Island/South Island juncture. J R Soc N Z 42:153–183

Trewick SA, Henderson IM, Pohe SR, Morgan-Richards M (2022) Spatial variation of *Acanthophlebia cruentata* (Ephemeroptera), a mayfly endemic to Te Ika-a-Māui—North Island of Aotearoa, New Zealand. Insects 13:567

Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Vasconcelos TN, Proença CE, Ahmad B, Aguilar DS, Aguilar R, Amorim BS, Campbell K, Costa IR, De-Carvalho PS, Faria JE (2017) Myrteae phylogeny, calibration, biogeography and diversification patterns: increased understanding in the most species rich tribe of myrtaceae. Mol Phylogenet Evol 109:113–137

Voelckel C, Heenan P, Janssen B, Reichelt M, Ford K, Hofmann R, Lockhart P (2008) Transcriptional and biochemical signatures of divergence in natural populations of two species of New Zealand alpine *Pachycladon*. Mol Ecol 17:4740–4753

Wallis GP, Trewick SA (2009) New Zealand phylogeography: evolution on a small continent. Mol Ecol 18:3548–3580

Wardle P (1963) Evolution and distribution of the New Zealand flora, as affected by quaternary climates. N Z J Bot 1:3–17

Wardle P (1991) Vegetation of New Zealand. Cambridge University Press, Cambridge, UK

Wessinger CA (2021) From pollen dispersal to plant diversification: genetic consequences of pollination mode. New Phytol 229:3125–3132

Westphal L (2019) Fruit Selection in New Zealand Avifauna: how dietary preference shapes mutaulisms Master's thesis, University of Canterbury

Wilmshurst JM, McGlone MS (1996) Forest disturbance in the central North Island, New Zealand, following the 1850 BP Taupo eruption. Holocene 6:399–411

Winter G (1884) Rabenhorstii fungi Europaei et Extraeuraopaei. Hedwigia 23:164–175

Wiser SK, De Cáceres M (2013) Updating vegetation classifications: an example with New Zealand's woody vegetation. J Veg Sci 24:80–93

Wiser SK, Hurst JM, Wright EF, Allen RB (2011) New Zealand's forest and shrubland communities: a quantitative classification based on a nationally representative plot network. Appl Veg Sci 14:506–523

Withers SJ, Parsons S, Hauber ME, Kendrick A, Lavery SD (2021) Genetic divergence between isolated populations of the North Island New Zealand rifleman (*Acanthisitta chloris granti*) implicates ancient biogeographic impacts rather than recent habitat fragmentation. Ecol Evol 11:5998–6014

Wood J, Wilmshurst J, Newnham R, McGlone M (2017) Evolution and ecological change during the New Zealand Quaternary. Landscape and quaternary environmental change in New Zealand. Springer. p.^pp. 235–291

Wright (1931) Evolution in Mendelian populations. Genetics 16:97–159

Yong WTL, Ades PK, Bossinger G, Runa FA, Sandhu KS, Potts BM, Tibbits JF (2019) Geographical patterns of variation in susceptibility of *Eucalyptus globulus* and *Eucalyptus obliqua* to myrtle rust. Tree Genet Genomes 15:1–14

Young AG, Schmidt-Adam G, Murray BG (2001) Genetic variation and structure of remnant stands of Pohutukawa (*Metrosideros excelsa*, Myrtaceae). N Z J Bot 39:133–140

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