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Conservation of two subspecies of *Olearia rugosa* (Asteraceae) post-bushfire complicated by occasional hybridisation

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Abstract

This study provides an overview of genetic diversity in populations of *Olearia rugosa* (F.Muell. ex Archer) Hutch. subsp. *angustifolia* Hawke ex Messina and subsp. *distalilobata* Hawke ex Messina, two of four Victorian subspecies, to assist in the development of a conservation framework. Populations of both subspecies burnt in the Black Summer bushfires (2019/2020) are genetically distinct from the two other Victorian subspecies. Hybridisation was confirmed at one site where *O. rugosa* subsp. *angustifolia* grows sympatrically with *O. lirata* (Sims) Hutch. and a morphological intergrade occurs there. Field identification based on morphology was inconsistent with genetic identity of species, subspecies and hybrids. *In situ* and *ex situ* genetic management including seed collection and genetic rescue require prior assessment of plant genetic status, particularly if there is a risk of hybridisation between taxa.

Keywords: isolated populations, hybridisation, *Olearia lirata*, DArTseq

Introduction

Geographical isolation and subsequent lack of gene flow between populations represents a major speciation factor in plants (Rieseberg & Willis 2007, Ellstrand 2014). Subspecies rank is commonly used in plant taxonomy to represent geographically isolated populations that exhibit significant and recognisable morphological differences, while remaining insufficiently distinctive to merit species status and retaining the potential to interbreed (Hamilton & Reichard 1992, Stajsic 2010, Messina *et al*. 2013, 2014). Despite some dispute about the legitimacy of accepting the rank of subspecies (Burbrink *et al.* 2022), their recognition can assist in promoting policy around conserving evolutionarily significant units (Braby *et al.* 2012) although the two are not necessarily equivalent. Genetic differentiation can be detectable between subspecies (Robins *et al*. 2021, Blyth *et al.* 2021, Sampson & Byrne 2022) and may be correlated with some reproductive isolation that may bring about speciation (Stankowski & Ravinet 2021). Genetic assessment is valuable for shaping conservation actions for species to ensure the unique genetic diversity of each taxon is recognised and preserved (Monks *et al.* 2022). This is particularly important if there are life strategies that differ between subspecies as this may impact how habitats are managed (Millar *et al*. 2010). Despite the value of genetic information in managing conservation targets, genetic evaluations have been included in only 52% of Australian species recovery plans (Pierson *et al*. 2016).

Hybridisation plays a significant role in speciation in many plant families including Asteraceae (Wong *et al*. 2022), both over evolutionary

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timeframes (Rieseberg *et al.* 2003) and in recent decades (Lowe and Abbott 2004, Abbott *et al.* 2009). Intra- and inter-specific hybridisation is common in plants (Griffin *et al*. 1988, Whitney *et al*. 2010) and has been observed to increase with environmental disturbance, whether natural (Stebbins 1950) or human-mediated, including the introduction of non-native species (Potts *et al.* 2003, Laikre *et al.* 2010, Larcombe *et al.* 2016). Negative risks of hybridisation include the loss of currently defined parental species identity and genetic uniqueness (Allendorf *et al.* 2001). For example, the introduced invasive species *Senecio madagascarensis* hybridises with the Australian native species *S. pinnatifolius,* the identity of which may come under threat if the presence of *S. madagascariensis* increases in areas of contact (Prentis *et al.* 2007, Dormontt *et al.* 2017). There is also the risk of outbreeding depression and genetic swamping with hybridisation, due to the disruption of local adaptation and decreased fitness in early generation hybrids (Lopez *et al.* 2000, summarised in Todesco *et al.* 2016). However, this must be balanced with the risks of inbreeding depression and a lack of genetic diversity that may impede adaptive potential and resilience, both of which could elevate the extinction risk for species in highly modified, altered, degraded and fragmented environments (Frankham 2015, Chan *et al.* 2019). Genetic traits gained through hybridisation can benefit a species by expanding its environmental tolerances and enabling greater ecological divergence than that of the parent species (e.g. *Helianthus*, Rieseberg *et al.* 2003) and may be beneficial for persistence under climate change (Vallejo-Marín *et al.* 2016, Pfeilsticker *et al.* 2022).

The evolutionary influence of hybridisation is variable (Dormontt *et al.* 2017, Mitchell *et al.* 2022) and the drivers of hybrid zones are still unknown for most species (Abbott 2017). Some authors have argued that novel hybrids may have a high conservation value because of their significance in evolution (Thompson *et al.* 2010), however, the conservation of novel hybrids and hybrid populations is complex (Muniz *et al.* 2020). In Australia, Commonwealth and state legislation do not afford consistent protection for hybrids (e.g. transient hybrids are afforded protection under the Victorian Government *Flora and Fauna Guarantee Act 1988* (FFG Act 1988) but not the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act 1999)). Similarly, conservation of hybrids may have detrimental flow-on effects for the conservation of native species where hybridisation with a foreign species result in new, fertile, stable hybrid taxa (Lowe & Abbott 2004, Abbott *et al.* 2009) rather than a transient hybrid entity (van Dijk *et al.* 2020). Moreover, hybridisation in plants can also impinge on the effectiveness of the conservation management of 'pure species' if not detected (Fatemi & Gross 2009), particularly in *ex situ* collections aimed to conserve the genetic distinctiveness and diversity of a species concept.

The genus *Olearia* as traditionally recognised, comprises ca. 180 species and is the most speciose genus of Asteraceae in Australia where all 130 species are endemic (VicFlora 2025). Unlike the majority of New Zealand species, the Australian taxa are noted for being largely diploid (2n=18), though there are exceptions, such as the *Olearia phlogopappa* (Labill.) DC. complex which is tetraploid (2n=36, Watanabe *et al.* 1996). *Olearia* is represented in Victoria by 43 species, 22 of which are included on the FFG Act 1988 (DELWP 2021, VicFlora 2025). Various authors have worked within several sections and complexes of *Olearia s.s.* (e.g. Hawke 2005, Messina *et al.* 2013, 2014), highlighting the difficulty non-botanists may have in accurately identifying species and indicating that further investigation is required to ascertain taxonomic delimitations. Although hybridisation has been reported in *Olearia* (e.g. Heenan 2005), there is little information on its frequency, the conditions that promote hybridity, or whether there are negative or positive consequences. Hybridisation has not been confirmed genetically in Australian taxa but morphological intergrades considered likely to be hybrids have been observed for various Australian species (e.g. *O. rugosa* (F.Muell. ex W.Archer) Hutch. subsp. *rugosa* and *O. lirata* (Sims) Hutch. in Victoria, and *O. phlogopapp*a and *O. lirata* in Tasmania (Messina *et al.* 2013; VicFlora 2025)). *Olearia rugosa* from southeastern Australia is comprised of five geographically disjunct subspecies. Three subspecies, endemic to Victoria, are narrowly distributed, often occurring in small, isolated populations (Messina *et al.* 2014). A fourth subspecies occurs only on Bass Strait islands and the fifth occurs mainly in Victoria but its range extends to southeastern NSW.

The entire ranges of two East Gippsland taxa, *O. rugosa* subsp. *angustifolia* Hawke ex Messina and *O. rugosa* subsp. *distalilobata* Hawke ex Messina, were affected by the Black Summer bushfires of 2019/2020. As a result, the two subspecies became post-bushfire targets for rapid assessment of survival and for the collection of seed for conservation purposes (Gallagher 2020, DCCEEW 2023). *Olearia rugosa* exhibits clear taxonomic delineation (see Messina *et al*. 2013) in contrast to the ambiguity observed in other congeners. Genetic diversity studies for the restricted subspecies of *O. rugosa* were proposed to develop *in situ* and *ex situ* conservation frameworks and to guide genetic rescue of small, isolated populations. Post-bushfire censuses found individuals exhibiting morphological intergrades in leaf shape and indumentum where *O. rugosa* subsp. *angustifolia* occurs with *O. lirata* in the western part of this subspecies' range, raising the possibility of hybridisation between these taxa. Confirming the genetic status of the intergrades would aid in ensuring that conservation seed collections comprise target taxa and not hybrid seed.

In this study, genetic data was used to directly benefit the conservation of at-risk subspecies of *O. rugosa*. Our primary aim was to examine genetic structure in two of the four Victorian subspecies, and to test for hybridisation in a population of *O. rugosa* subsp. *angustifolia* growing sympatrically with *O. lirata*, where morphological intermediates were observed. This information can be used to prioritise actions that preserve genetic diversity *in situ* and guide sampling to maximise the genetic diversity of target taxa in *ex situ* collections.

Methods

Species background

Olearia rugosa is confined largely to Victoria, growing as a spindly shrub to 2.5 m high, with green, wrinkled leaves that are tuberculate above and stellate-tomentose below, with white ray florets surrounding yellow or purplish disc florets. There are five morphologically and geographically discrete subspecies, four of which occur in Victoria. Of those, two were affected by the Black Summer bushfires (Figure 1).

Olearia rugosa subsp. *allenderae* (J.H.Willis) Hawke ex Messina is restricted to swampy areas or gully slopes in Wilsons Promontory National Park, Victoria, and is listed as Endangered (EN) under the FFG Act (VicFlora 2025). Leaves of this subspecies are elliptic, ovate or narrow-triangular with deeply serrate to crenate margins.

Olearia rugosa subsp. *angustifolia* is naturally rare and often forms quite small (<20 plants), localised populations near streams and swamps, or on sandy rises in near-coastal areas of the East Gippsland lowlands. This subspecies is distinguished by leaves that are more

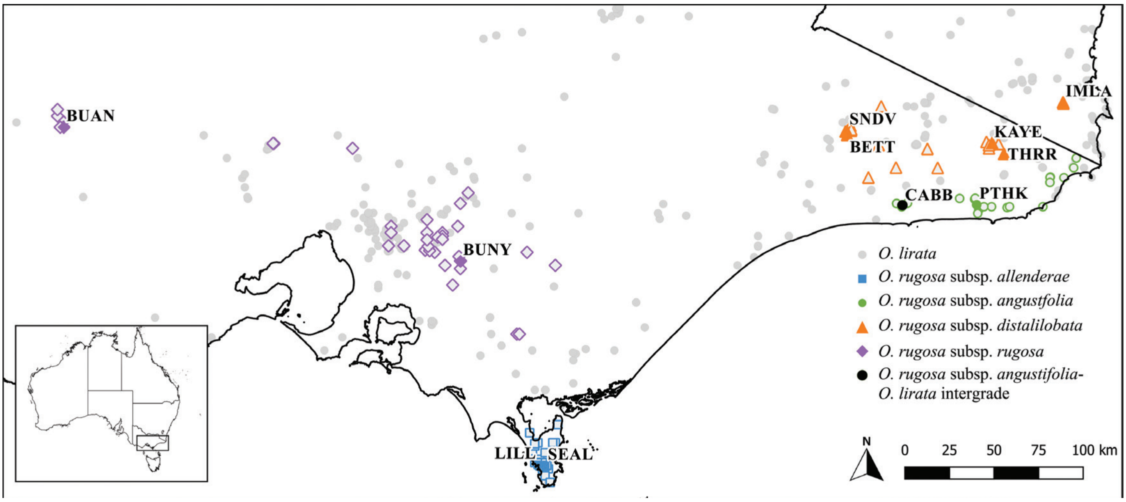

Figure 1. Four subspecies of *Olearia rugosa* and *O. lirata* in Victoria and southeast NSW with hollow symbols showing the overall distribution of each according to herbarium collections (AVH 2025) and filled symbols indicating locations sampled for this study. Colours are given in legend. Population codes are provided in Table 1, with CABB showing the location of CABBA, *O. rugosa* subsp. *angustifolia*, CABBL *O. lirata* and CABBU morphological intergrade collections.

or less linear with serrate to crenate margins and it is a candidate for FFG nomination given its geographic and demographic restriction. With the exception of Cabbage Tree Creek, all known locations in Croajingalong National Park were affected by the Black Summer bushfire. During post-bushfire surveys for this study, morphological intergrades were observed between *Olearia rugosa* subsp. *angustifolia* and sympatric *O. lirata* at Cabbage Tree Creek.

Olearia rugosa subsp. *distalilobata* occurs on rocky gully slopes of montane forest in remote areas from the Snowy River through to Mt Imlay in southeast NSW. This species is distinguished by its elliptic to oblong or obovate leaves with subentire or distally crenate margins. Eight populations were originally known and several factors, including logging, are likely to have caused local extinction at three locations in the last 30 years. This subspecies is listed as Endangered (EN) under the EPBC Act (1999) and five locations were affected by the Black Summer bushfire (DELWP 2022).

Olearia rugosa subsp. *intermedia* Messina is restricted to islands in Bass Strait and northeastern Tasmania. As the species epithet suggests, this species is characterised by a somewhat intermediate leaf shape (VicFlora 2025).

Finally, *Olearia rugosa* subsp. *rugosa* is distinguished by leaves that are ovate, elliptic or suborbicular with margins lobed throughout. It is the most widespread subspecies, occurring in wet to damp forest on southern slopes of the Dividing Range from Mount Macedon to Drouin, with isolated records from Mount Buangor and the Strzelecki Ranges. Possible hybrids and morphological intergrades with the widespread species *Olearia lirata* (Sims) Hutch. have been observed occasionally where these two species co-occur in the Beenak – Warburton area to the northeast of Melbourne (VicFlora 2025). *Olearia lirata* is a highly plastic congener that can occur sympatrically with all subspecies of *O. rugosa* from locations near sea-level to subalpine areas, in moist forest and scrub throughout southeastern Australia.

The four Victorian subspecies and one population of *O. lirata* were sampled. The two subspecies affected by the Black Summer bushfires, *Olearia rugosa* subsp. *angustifolia* and *O. rugosa* subsp. *distalilobata,* were the focus of this work and were sampled across their ranges. Two populations each were sampled for *O. rugosa* subsp. *allenderae* (highly restricted) and subsp. *rugosa* (more widespread) to assess genetic boundaries between subspecies. *Olearia rugosa* subsp. *intermedia* was not included in this study due to the significant geographic disjunction from the Victorian subspecies.

Sampling

Field surveys targeted locations of *Olearia rugosa* subsp. *distalilobata* and subsp. *angustifolia* that were prioritised for action by the Department of Climate Change, Energy, the Environment and Water (DCCEEW 2023). In total, 11 locations were sampled for DNA analysis (Figure 1; Table 1) based on National Herbarium

Table 1. Location and sampling details for *Olearia* used in this study including the dataset the samples were used in. Populations marked with an asterisk were affected by widespread bushfires in 2019/2020 and all plants sampled at these locations were seedlings up to two years old. Adult plants were sampled at all other locations.

Taxon	Location	Code	# samples	used in dataset
O. rugosa subsp. *rugosa*	Buangor	BUAN	5	1
O. rugosa subsp. *rugosa*	Bunyip	BUNY	5	1
O. rugosa subsp. *angustifolia*	Point Hicks	PTHK*	5	1, 2
O. rugosa subsp. *angustifolia*	Cabbage Tree Creek	CABBA	5	1, 2
O. lirata		CABBL	5	1, 2
morphological intergrade		CABBU	16	1, 2
O. rugosa subsp. *distalilobata*	Snowy Divide Track	SNDV*	8	1
O. rugosa subsp. *distalilobata*	Tulloch Ard	BETT*	5	1
O. rugosa subsp. *distalilobata*	Thurra River	THRR	5	1
O. rugosa subsp. *distalilobata*	Mt Kaye	KAYE*	5	1
O. rugosa subsp. *distalilobata*	Mt Imlay	IMLA*	5	1
O. rugosa subsp. *allenderae*	Lilly Pilly Track	LILL	5	1
O. rugosa subsp. *allenderae*	Sealers Cove Track	SEAL	9	1

of Victoria specimens or Atlas of Living Australia observation records (Australasian Virtual Herbarium 2022) with the Cabbage Tree Creek location (CABB) comprising three separate collections. Five plants were sampled for each taxon at each location unless stated otherwise. In all locations, samples were gathered from plants believed to be separate individuals spaced at least 10 m apart across the geographic extent of the given population. Populations marked with an asterisk (Table 1) were affected by widespread bushfires in 2019/2020 and all plants sampled at these locations were seedlings up to two years old. Adult plants were sampled at all other locations. For all populations, a representative herbarium sample was collected for the National Herbarium of Victoria.

Olearia rugosa subsp. *distalilobata* was collected from four locations in Victoria (THRR, SNDV, BETT and KAYE), and one location in NSW (IMLA). Several historic collection locations for this subspecies in Errinundra and Snowy River National Parks are considered extinct. No attempts were made to re-survey these locations (DCCEEW 2023).

Olearia rugosa subsp. *angustifolia* was collected from only two sites, PTHK and CABB. No plants were found at Miners Track and the status of plants at the following locations is unknown as sites have been inaccessible for several seasons due to damage from bushfires and flooding: Cicada Trail, Benedore River Mouth, and Spotted Dog Mine. At Cabbage Tree Creek (CABB), plants of intermediate leaf shape and indumentum morphology were observed intergrading from typical subsp. *angustifolia* through to typical *O. lirata* and were considered possible hybrids between the two taxa. Therefore, in addition to five samples collected from plants identified morphologically as pure subsp. *angustifolia* (CABBA), samples were collected from 16 plants that varied across a morphological gradient from subsp. *angustifolia* to *O. lirata* (CABBU). An additional five samples, considered to be pure *O. lirata* based on morphology, were collected approximately 50 m away from the putative hybrid population (CABBL). Morphology was broadly assigned in the field then verified under the microscope upon return from the field. In contrast, no intergrades were observed at PTHK despite the presence of both *O. rugosa* subsp. *angustifolia* and *O. lirata*.

Samples were included from two sites each of *O. rugosa* subsp. *allenderae* from Wilsons Promontory (LILL and SEAL) and *O. rugosa* subsp. *rugosa* from Buangor (BUAN) and Bunyip State Forest (BUNY).

DNA extraction, amplification and sequencing

Silica-dried leaf samples (83 samples, 11 technical repeats = 9 samples including technical repeats for each taxon) were sent to the commercial genotyping service, Diversity Arrays Technology ('DArT', Canberra, Australia) for DNA extraction and DArTseq^{TM} analysis, a reduced representation sequencing method (Kilian *et al.* 2012). DNA was extracted at DArT using the Nucleo Mag kit (Machery Nagel, Germany) on a Tecan 100 platform following the manufacturers protocols. Library preparation involved DNA digestion and ligation using methylation-sensitive restriction enzymes PstI and MseI and uniquely barcoded adaptors. Following PCR and quantification, the samples were standardised and pooled for sequencing in a single lane of an Illumina NovaSeq X+ sequencer. Sequences were processed using proprietary DArT analytical pipelines to filter poor quality sequences. Resultant sequences were used in DArTsoft14, DArT PL's proprietary SNP calling algorithms.

Data analysis

The unfiltered SNP dataset received from DArT consisted of 69,329 SNPs across 93 samples, with one sample failing sequencing (*O. rugosa* subsp. *distalilobata* from THRR). Initial screening for clones (genetically indistinguishable samples) was made by separating the dataset into broad taxonomic groups and using Euclidean genetic distance calculations via the R packages dartR (Gruber *et al.* 2018) and Poppr (Kamvar *et al.* 2014, 2015). All analyses were conducted in R (R Core Team 2019) version 4.4.1 unless otherwise specified. A threshold to select clonal samples was calculated as the average value between technical repeat pairs plus/minus three standard deviations of the repeat pair values. Any sample pairs below these values in the genetic distance measure were considered clonal samples. A kinship analysis using PLINK method of moment (MoM) for the Identity-By-Descent (IBD), analysis in SNPRelate (snpgdsIBDMoM; Zheng *et al.* 2012) was used to verify monoclonal individuals. Clonal samples were identified in some but not all populations of *O. rugosa* subsp. *distalilobata* (detailed below) which hindered balanced calculation of heterozygosity statistics for populations, so they are not reported here. Technical replicates were removed from all further datasets and analyses.

Dataset 1 was used to initially visualise relationships among species, subspecies and populations and included 82 samples, including clonal samples, across all 11 locations sampled. To enhance the data quality yet retain SNPs across broader phylogenetic groups, Dataset 1 was filtered for secondaries, read depth (5-90), a reproducibility threshold of 0.95, loci call rate of 0.4, individual call rate of 0.45, filtered for Hamming distance to remove possible paralogues and monomorphic loci using the R package dartR (Gruber *et al.* 2018). One *O. rugosa* subsp. *rugosa* individual from BUNY was removed in these steps, with the resultant Dataset 1 comprised of 13,261 SNPs across 81 individuals. A phylogenetic network was constructed with the NeighborNet method using the R package RSplitsTree (ver. 0.1.0, see http://rdrr.io/github/ IVS-UZH/RSplitsTree/) a GENPOFAD distance metric suitable for SNP markers, calculated in the 'pofadinr' R package (Joly *et al.* 2015) and visualised in R using ggplot2 (ver. 3.4.2; Wickham 2016) and tanggle (ver. 1.0.0, see http://rdrr.io/github/ KlausVigo/tanggle/). Unlike bifurcating trees, phylogenetic networks can capture complex evolutionary histories (e.g. reticulation events) and thus may be more informative when assessing relationships among closely related species as well as infraspecific relationships (Huson and Bryant 2006).

Dataset 2 was used to interrogate the morphological intergrade observed at the CABB location and included all samples from the CABB location including *O. lirata* samples from this location and the *O. rugosa* subsp. *angustifolia* from PTHK. The raw dataset of 36,708 SNPs across 31 individuals was filtered as described above with the loci call rate set at 0.8, individual call rate of 0.57 and MAF set to 3% to give final Dataset 2 composed of 3,910 SNPs and 31 individuals. A principal component analysis (PCA) implemented in the adegenet package (Jombart, 2008; Jombart & Ahmed, 2011) in R (R Core Team, 2019) was used to visualise introgression between taxa across the Cabbage Tree Creek population. A Bayesian analysis implemented in STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000) was used to identify K genetic clusters in the dataset without any information regarding taxon assignment or geographic proximity. The upper K limit was set to five on the assumption that there could be four potential genetic groupings. Ten independent runs were undertaken for each K value with a burn-in of 100,000 and 200,000 MCMC iterations. The R package pophelper (Francis 2017) and ggplot2 (Wickham 2016) were used to visualise results and select the most probable K based on the ΔK metric (Evanno *et al.* 2005).

The *gl.fixed.diff* function was utilised on Dataset 2 in dartR to export the top 200 loci (by call rate) exhibiting a fixed difference between the putative parental taxa using dartR package for use in a hybrid analysis (Gruber *et al.* 2018). Putative parent taxa in CABB were assigned based on morphological identification as either *z0* or *z1*, with samples from PTHK assigned *s*, pure origin *O. rugosa* subsp. *angustifolia* that were not to be considered part of the mixture relevant to the analysis. NewHybrids Version 1.1 beta (Anderson & Thompson 2002; Anderson 2003) was used to estimate the posterior probability (range 0–1) that individuals fall into pure parental, F_1 hybrids, F_2 hybrids or first-generation backcrosses based on the genotype frequency classes of the 200 selected loci, the Jeffreys Prior for θ and 10,000 burnin with 90,000 MCMC sweeps. The results from this analysis were used to assign a probability that each sample from CABB was either a parent taxon (*O. rugosa* subsp. *angustifolia*, *O. lirata*), hybrid taxon (F_1 or F_2 *O. rugosa* subsp. *angustifolia* x *O. lirata*) or a backcrossed hybrid taxon with either parent.

Results

The raw data from DArTSeq contained 93 samples and 68,672 loci. The clonal analysis found genetically indistinguishable samples within several *O. rugosa* subsp. *distalilobata* populations, five samples within IMLA, two samples within KAYE and three samples within THRR. No other clones were identified within any of the taxonomic group datasets. After filtering as outlined above, each of the final SNP data sets in the analysis were the following dimensions: Dataset 1 contained 13,261 SNPs and 81 individuals across 11 locations and Dataset 2 contained 3,910 SNPs and 31 individuals across two locations.

Species structure

The NeighborNet phylogenetic network from Dataset 1 visually displays the morphological intergrade observed at the CABB location with individuals identified as *O. rugosa* subsp. *angustifolia* and *O. rugosa* subsp. *angustifolia* x *O. lirata* hybrids and *O. lirata* from this location in one cluster (Figure 2). Two pairs of populations, BETT and SNDV (*O. rugosa* subsp. *distililobata*) and SEAL and LILL (*O. rugosa* subsp. *allenderae*), are connected at the base of the branches reflecting the historical genetic reticulation occurring due to the close geographic proximity of each pair. All other populations of each subspecies were clearly differentiated with many populations, including IMLA and BUAN, forming monophyletic clades on long branches showing little/no reticulation with neighbouring populations of the same subspecies.

Hybridisation within Cabbage Tree Creek

The PCA based on Dataset 2 provides higher resolution of the CABB population with axes 1 and 2 accounting for 28.49% of the variation in the dataset. The species are separated across axis 1 with *O. lirata* (CABBL; squares) in the negative space and *O. rugosa* subsp. *angustifolia* from CABBA (circles) and PTHK (triangles) in the positive space (Figure 3a). The morphological intergrade

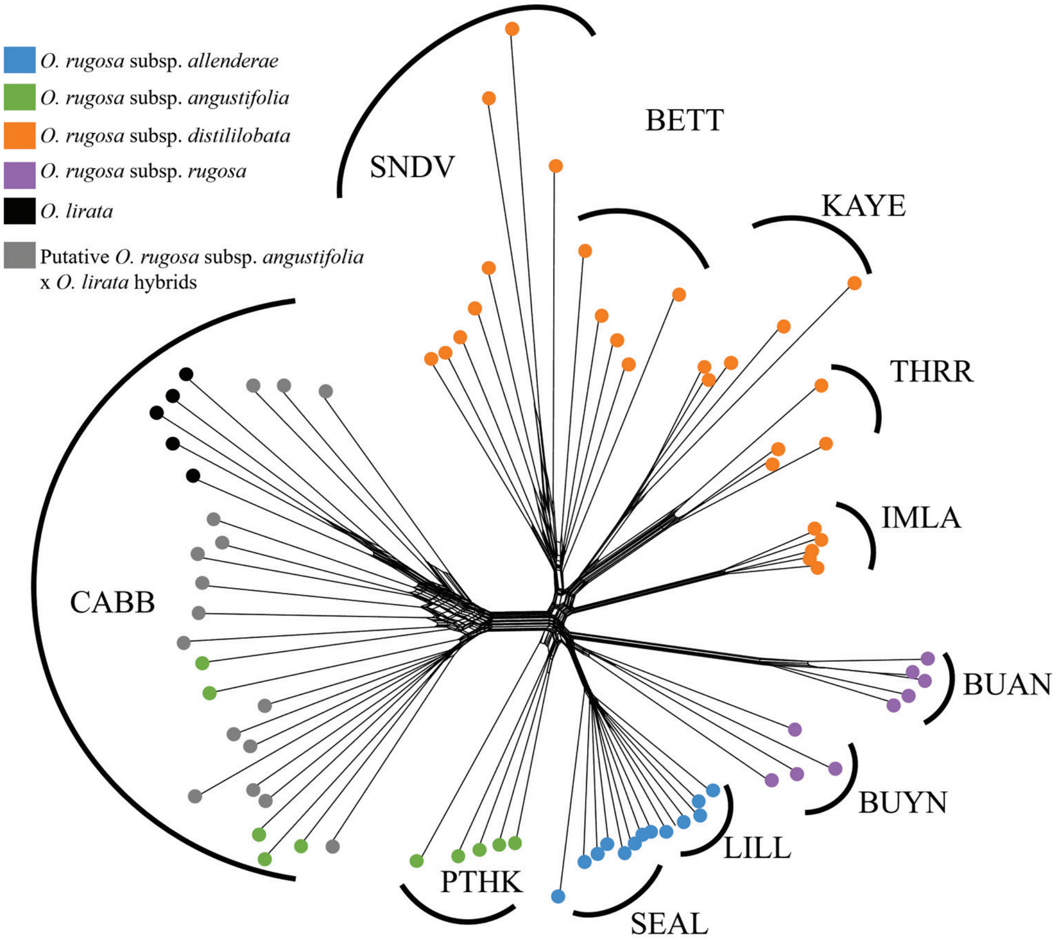

Figure 2. Phylogenetic network based on GENPOFAD distance 13,261 SNPs and 81 individuals in *Olearia* Dataset 1 labelled by their population code and sample name, coloured by species as identified when collected. Black = *O. lirata*, olive green = *O. rugosa* subsp. *angustifolia*, grey = *O. lirata* × *O. rugosa* subsp. *angustifolia* morphological intergrade, orange = *O. rugosa* subsp. *distalilobata*, blue = *O. rugosa* subsp. *allenderae*, purple = *O. rugosa* subsp. *rugosa*.

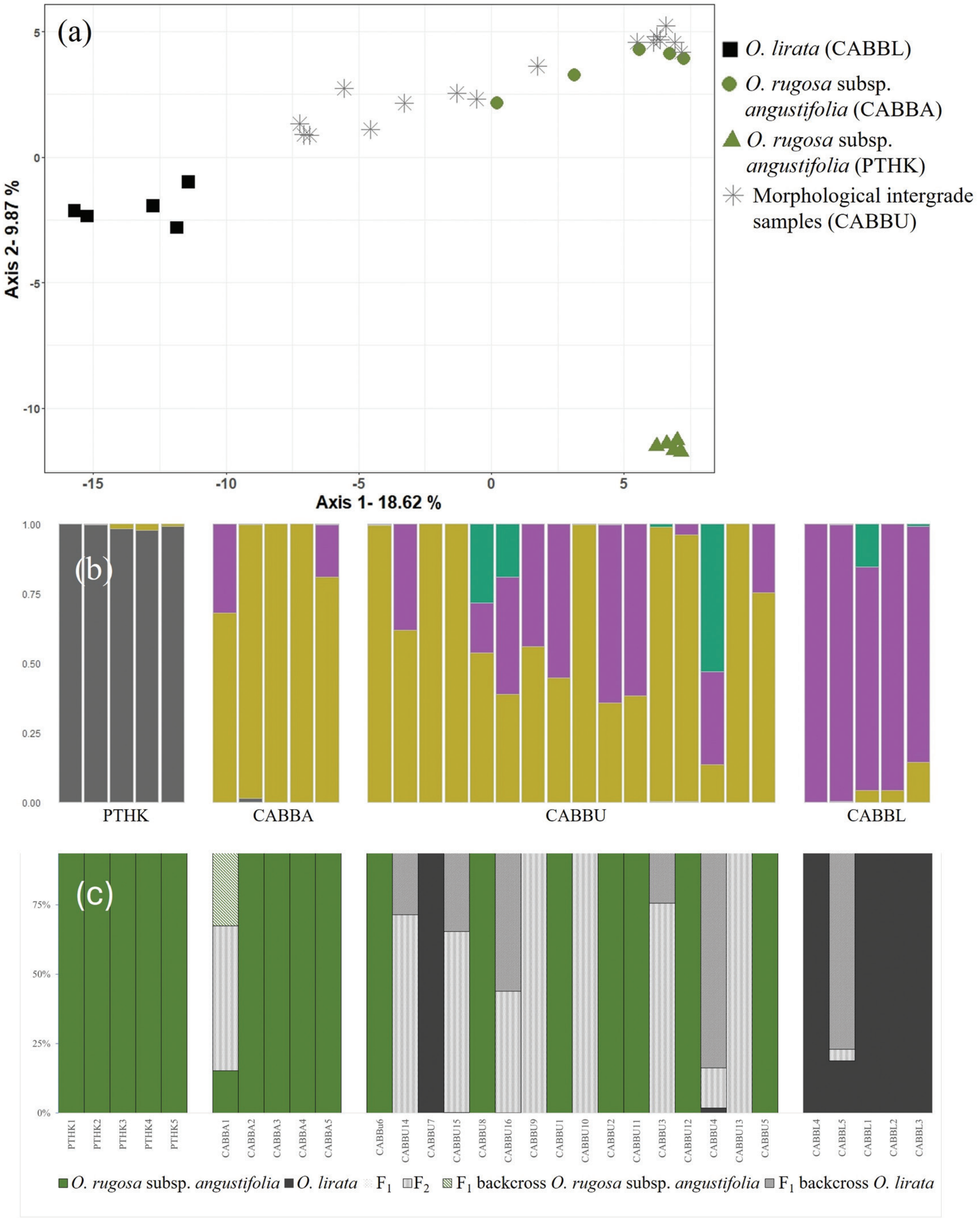

Figure 3. Results of genomic analysis of 31 *Olearia* individuals including *O. rugosa* subsp. *angustifolia*, *O. lirata* and morphological intergrades between these taxa from the CABB and PTHK locations based on Dataset 2 (3,910 SNPs). (a) first and second axis of the principal component analysis; individual samples are coloured by species and shapes indicate population of origin as detailed in legend. (b) STRUCTURE results displaying barchart of individual Q-membership proportions to each of K=4 clusters, with individuals clustered by population. Population labels apply to the chart displayed in (c). (c) The plot of the posterior probabilities of the genotype class estimated with NewHybrids with the proportion of each individual assigned to either pure species, F_1, F_2 hybrids or F_1-backcross. Individual labels below (c) apply to the chart displayed in (b).

samples from CABB (stars) placed intermediately (Figure 3a). Some *O. rugosa* subsp. *angustifolia* samples are placed towards the intermediate space and some of the morphological intergrade samples are placed closer to *O. rugosa* subsp. *angustifolia,* potentially suggesting backcrossing to this parent taxon. The *O. rugosa* subsp. *angustifolia* populations PTHK and CABB are separated across axis 2.

The Evanno ΔK method identified K=4 genetic clusters from the Structure analysis (Figure 3b). The morphologically typical *O. rugosa* subsp. *angustifolia* samples from the PTHK location were primarily composed of cluster 1 (grey). Cluster 2 primarily aligned with the *O. lirata* individuals from CABBL (purple). The *O. rugosa* subsp. *angustifolia* individuals from CABBA were aligned with a third cluster (yellow) although two individuals from this population showed admixture with cluster 2 (Figure 3b). The morphological intergrade individuals (CABBU) had several individuals that were either entirely made up of the third cluster (5) or primarily this cluster with a very small amount of admixture (2), five were admixed primarily cluster 3 with a portion assigned to cluster 2, while three others exhibited admixture between clusters 2, 3 and 4.

The results of the New Hybrids analysis provide further resolution on the admixture present at the CABB location and for the most part is consistent with the Structure results (Figure 3c). Four of the five *O. rugosa* subsp. *angustifolia* individuals (CABBA) were identified as pure species while one individual had similar probability assignment as an F_2 hybrid or a F_1-backcross with *O. rugosa* subsp. *angustifolia* (Figure 3c; Table 2). The *O. lirata* samples (CABBL) had a similar pattern with four individuals identified as pure *O. lirata* and one with a high probability of a F_1-backcross with *O. lirata*. The morphological intergrade samples (CABBU) ranged from a high probability of pure *O. rugosa* subsp. *angustifolia* (7) and pure *O. lirata* (1), to F_2 individuals (3), with the remaining five individuals F_1-backcrossed with *O. lirata*. One notable incongruence with the Structure results was the assignment of CABBU7 as pure *O. lirata* in the NewHybrids analysis while in the Structure results, this individual was assigned to cluster 3 (CABBA group). Of additional interest is an absence of F_1 hybrids. Of the 26 individuals from the CABB location (CABBA, CABBU, CABBL), only 14 had a field identification that was consistent with the identification from genomic analysis (Table 2).

Discussion

The genus *Olearia* is one of the largest Asteraceae genera and this work adds to the number of conservation genetic studies made of threatened *Olearia* species (Smith *et al.* 2004, Barnaud & Houliston 2010) including those in the genomics era (Blyth *et al.* 2021). This genomic work has characterised the distinctiveness of populations within four of the five subspecies of *O. rugosa*, including *O. rugosa* subsp. *distalilobata,* listed under the federal EPBC Act, and *O. rugosa* subsp. *angustifolia,* listed under the Victorian FFG Act. Most populations assessed in this work were small and isolated, as such the barriers to connectivity between populations are evidenced in the lack of reticulation between populations in the NeighborNet analysis. A hybrid swarm, defined here as a morphologically variable group of plants consisting of parental species, hybrids and crosses among them, that was quantified in one population of *O. rugosa* subsp. *angustifolia* highlights the permeable nature of species boundaries and raises complications for conservation listings and *ex situ* conservation collections.

Genetic structure and diversity

The structure of the phylogenetic network reflects the disjunct range of *O. rugosa*. Some populations of *O. rugosa* subsp. *angustifolia* and *O. rugosa* subsp. *distalilobata* are separated by more than 30 km. Even if continuous forest exists, gene flow would be limited between populations of each subspecies, resulting in genetic drift between populations over time. Similar patterns have been found for other threatened *Olearia* species (Smith *et al.* 2004, Blyth *et al.* 2021) and other endangered Asteraceae species (Rodger *et al.* 2021). Fire tolerance is not well understood for subspecies of *O. rugosa* and may have differing demographic and, consequently, genetic impacts depending on whether a species is a resprouter or obligate seeder. Obligate seeders are susceptible to local population extinctions when fire intervals are shorter than the time needed to re-establish seedbanks (Whelan 2002, Clarke *et al.* 2015) which could lead to a dramatic loss of genetic diversity. Resprouting enables the standing numbers of a species to rebound quickly, avoiding the loss of genetic diversity and a reliance on a seedbank. However, sexual reproduction is important as it enables the production of genetically variable seed and novel genotypes for landscape persistence, essential for longer term evolution and adaptation to changing environments (Bürger 1999). Little is known of the comparative phenology, reproductive biology and pollinators of the

Table 2. *Olearia* individuals in Dataset 2 with the degree of hybridisation observed by morphological identification in the field and posterior probabilities of the genotype class estimated by NewHybrids.

Individual	Morphological ID	NewHybrids Result
Point Hicks (PTHK)		
PTHK1	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
PTHK2	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
PTHK3	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
PTHK4	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
PTHK5	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
Cabbage Tree Creek (CABB)		
CABBA1	*Olearia rugosa* subsp. *angustifolia*	50% F_2, 30% F_1 backcross *angustifolia*, 20% *angustifolia*
CABBA2	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
CABBA3	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
CABBA4	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
CABBA5	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
CABBU1	*Olearia lirata*	*Olearia rugosa* subsp. *angustifolia*
CABBU2	Hybrid	*Olearia rugosa* subsp. *angustifolia*
CABBU3	Hybrid	80% F_2, 20% F_1 backcross *O. lirata*
CABBU4	*Olearia lirata*	80% F_1 backcross *O. lirata*, 20% F_2
CABBU5	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
CABBU6	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
CABBU7	*Olearia lirata*	*Olearia lirata*
CABBU8	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
CABBU9	*Olearia rugosa* subsp. *angustifolia*	F_2
CABBU10	*Olearia lirata* or hybrid	F_2
CABBU11	*Olearia lirata*	*Olearia rugosa* subsp. *angustifolia*
CABBU12	*Olearia rugosa* subsp. *angustifolia*	*Olearia rugosa* subsp. *angustifolia*
CABBU13	*Olearia lirata* or hybrid	F_2
CABBU14	*Olearia lirata*	70% F_2, 30% F_1 backcross *O. lirata*
CABBU15	*Olearia lirata* or hybrid	70% F_2, 30% F_1 backcross *O. lirata*
CABBU16	*Olearia lirata*	60% F_1 backcross *O. lirata*, 40% F_2
CABBL1	*Olearia lirata*	*Olearia lirata*
CABBL2	*Olearia lirata*	*Olearia lirata*
CABBL3	*Olearia lirata*	*Olearia lirata*
CABBL4	*Olearia lirata*	*Olearia lirata*
CABBL5	*Olearia lirata*	80% F_1 backcross *O. lirata*, 20% *O. lirata*

species. Plants are visited by a range of insects, which may or may not pollinate it, and there is no data on the species ability to self-pollinate.

A limitation of this study was the small numbers of samples collected from each location and an inability to compare genetic diversity results between populations due to the high levels of genetically indistinguishable samples in several *O. rugosa* subsp. *distalilobata* populations. Post-bushfire observations documented seedlings of *O. rugosa* subsp. *angustifolia,* while both seedlings and resprouting were documented in *O. rugosa* subsp. *distalilobata* (DELWP 2022). For the latter subspecies, resprouting was observed after high severity fire at Mt Imlay (IMLA) but the population suffered a decline in the number of live plants with only 42% of previously recorded plants observed to be resprouting (DCCEEW 2023). Thus, the identical genotypes observed at the IMLA, KAYE and THRR populations may be a result of sampling multi-stemmed resprouting individuals in the landscape. Additional detailed population demographic surveys and genetic studies, particularly at the IMLA location would assist in quantifying clonality and identifying levels of genetic diversity across the subspecies on the summit of this isolated mountain as genetic rescue may be an important component of conservation actions at this location. If an absence of multi-stemmed resprouting individuals is documented at the populations with a high number of clonal genotypes (IMLA, KAYE and THRR), investigations into mating systems may be necessary to eliminate the possibility of apomixis occurring within these populations as this would significantly alter the available genetic diversity at each site.

Hybrid zone and implications for conservation

Increasingly genetically diverse *ex situ* conservation seed bank collections are playing strong roles in the conservation of—and as a germplasm source for—the restoration of species after events that may dramatically affect remnant populations (IUCN/SSC 2014, Martyn Yenson *et al.* 2021). During post-bushfire surveys for *O. rugosa* subsp. a*ngustifolia,* morphological intergrades between *O. rugosa* subsp. *angustifolia* and the more common *O. lirata* were observed at the CABB location. Some incongruence was noted between the results of the Structure and NewHybrids analyses. Structure detects admixture by gene flow between populations over time and for this study was based on the full dataset of 3,910 SNPs across all 31 individuals, while NewHybrids uses a subset of 200 SNPs to identify the probability that an individual is classified as either a pure parent, F_1, F_2 or backcross taxon. While the latter analysis can be sensitive to sample sizes and is limited to identifying specific hybrid classes, it is still useful in identifying cryptic hybrids that may not be morphologically distinguishable. The combined PCA, Structure and NewHybrids analyses identified that there is indeed an *O. rugosa* subsp. *angustifolia* × *O. lirata* hybrid swarm that occurs at CABB. At this point in time, it is unknown if hybridisation at CABB has been ongoing or was a rare event triggered by an environmental change. However, the sampling in this work did not identify any F_1 hybrids present in the CABB location, which could be either because they were not sampled or because F_1 hybrid plants have died out as F_2 hybrids ($F_1 \times F_1$ crosses) have been recruited in the population. A level of hybrid fertility is suggested by the presence of F_2 hybrids and F_1 backcrosses to one or the other parental species, suggesting hybridisation has been occurring here at least for several generations. The CABB location was not affected by the Black Summer fire and is an intersection between a lowland forest and a damp forest vegetation community which has a tolerable fire interval of greater than 25 years and has thus been excluded from contemporary fuel management programs (Cheal 2010). Therefore, it is entirely possible that putative hybrids identified in this study are from a chance opportunity where the boundary between the two forest types that supports the parental forms have met and the resultant putative hybrids have variable fitness in comparison to the parents (Harrison & Rand 1989). No other hybrid zones were identified visually at post-bushfire survey locations of *O. rugosa* subsp. *angustifolia* (PTHK and at the Miners Track location, which was not included in this study) despite *O. lirata* being recorded at PTHK.

While attempts were made to identify 'pure' individuals using morphology and keys during and after field collecting trips, the data presented here found that only 14 of the 26 field identifications assigned across a spectrum from pure *O. rugosa* subsp. *angustifolia* through hybrids to pure *O. lirata* were consistent with the hybrid identification gained from genomic analysis. It is therefore preferable for *ex situ* germplasm conservation collections to target alternative *O. rugosa* subsp. *angustifolia* populations to ensure that hybrid germplasm is not unknowingly stored or used in potential population enhancements. This highlights the morphological complexities that can arise in hybrid swarms. In many cases, only early generation hybrids are likely to be recognised morphologically because later generation hybrids and backcrosses resembling one or both parents will be identified as such (Abbott 2017, Gawrońska *et al.* 2023). Consequently, *O. rugosa* subsp. *angustifolia* × *O. lirata* hybrid zones comprising backcrosses may not have been recognised at other wild sites based on morphological analysis alone. Additional surveys and sampling from other *O. rugosa* subsp. *angustifolia* locations when accessible would assist in quantifying how often *O. rugosa* subsp. *angustifolia* and *O. lirata* occur sympatrically, whether morphological intergrades are present and the extent of hybridisation when the two are present. It would be useful to expand such work to include other subspecies of *O. rugosa* given that possible hybrids and morphological intergrades have been observed occasionally where *O. rugosa* subsp. *rugosa* and *O. lirata* co-occur in the Beenak – Warburton area to the northeast of Melbourne (VicFlora 2025). Examining breeding system, seed set and pollen viability across all morphological intergrades may help understand the evolutionary history and future of such populations.

A valuable outcome of this study was that surveys struggled to locate many historical populations of *O. rugosa* subsp. *angustifolia*. Being a hybrid swarm, CABB is possibly composed of very few 'pure' *O. rugosa* subsp. *angustifolia* individuals. Based on the relatively narrow range, the disjunction between populations, small population sizes, the likely reduction in both the number and size of populations, as well as the ongoing threat of growing along roadsides, this subspecies is worthy of consideration for assessment against IUCN criteria and would likely meet Endangered given the similarity to *O. rugosa* subsp. *distalilobata*. The IUCN assessment of this subspecies would need to take into consideration the fact that the size of the 'pure' *O. rugosa* subsp. *angustifolia* population at CABB is potentially only half of what is observable by morphology. An additional impediment to a rapid listing would be the need to survey at very isolated historical locations (VicFlora 2025), as well as at sites such as Miners Track where the species was initially found immediately post bushfire but not re-located thereafter. While the coastal lowland habitat is a mosaic of vegetation types with minimal land use change, several decades have passed since the species was previously recorded at some locations and the long-term survival of the species post-bushfire is unknown.

Hybridisation is a 'double-edged sword' for closely related species or subspecies which interbreed frequently, particularly if one taxon is considered rarer or has a competitive advantage over the other. Genetic swamping and introgression could lead to the local extinction of one or the other taxa or, for highly localised taxa, the extinction of entire lineages. On the flip side, the beneficial impacts of hybridisation include the provision of increased genetic diversity and negation of inbreeding depression, as well as driving the evolution of new, reproductively isolated species through homoploid hybrid speciation where there is no change in chromosome number, or allopolyploid speciation where increasing ploidy levels may improve or restore hybrid fertility (Schley *et al.* 2022; Todesco *et al.* 2016). Furthermore, ecological and genetic studies as well as quantification of ploidy within the various entities of the *O. rugosa* subsp. *angustifolia* hybrid swarm in the CABB population would be valuable for elucidating the taxon's conservation value from an evolutionary point of view. 'Stable' hybrid entities that maintain distinct hybrid morphology are afforded protection under the Commonwealth EPBC Act and the Victorian FFG 1988. However, under both Acts, there are no specified genetic or morphological criteria that must be satisfied for a hybrid entity to be deemed stable.

The Victorian Conservation Seedbank (VCS) holds seed collections for *O. rugosa* subsp. *angustifolia* from the PTHK location and for *O. rugosa* subsp. *distalilobata* from the BETT location. Additional collections of *O. rugosa* subsp. *distalilobata* from the KAYE and THRR locations and of *O. rugosa* subsp. *angustifolia* from any other population of this subspecies would enhance the representation of genetic diversity held *ex situ* given the genetic differentiation identified between locations assessed in this study. For collection at locations such as

CABB, where *O. rugosa* subsp. *angustifolia* and *O. lirata* grow sympatrically, caution should be given to the conservation value of the seed given the potential for hybrid seed. *Olearia rugosa* subsp. *distalilobata* can be propagated reliably from cuttings and therefore this may be an alternative for *ex situ* conservation of the species if care is taken to capture a diverse group of genotypes (McDougall *et al*. 2023). Forest fire interval planning should allow time for plants to reach maturity so that seed may accumulate in the soil seedbank to maintain genetic diversity over time.

Conservation of threatened species faces many challenges and, in the case of *Olearia rugosa* subspecies, the potential for hybridisation can complicate efforts to preserve taxa (Fitzpatrick *et al.* 2015), especially where hybridisation may be facilitated by human-mediated changes (Ottenburghs 2021). Combined with further genetic divergence leading to speciation of subspecies (Garmendia *et al.* 2022), this poses a conundrum in terms of deciding what to protect, particularly when remnant populations are few and small, a challenge that is increasingly encountered when making conservation decisions. If, as is the case here, there are few remaining populations, one is obliged to consider whether to engage in conservation actions aimed at preserving the "pure" genetics at the population, or to avoid interference with natural evolutionary processes (Genovart 2009). If the hybrid is outcompeting the parental genotypes and thrives over time to become a new taxon that is better suited to the conditions (e.g. Riesberg *et al.* 2003*)*, should resources be invested into preserving a moment in time along the evolutionary continuum? It may be advisable to develop a conservation strategy that incorporates hybrid populations as they may be a source of novel genetic diversity with potential adaptive advantages (Thompson *et al.* 2010). This could be facilitated by incorporating seed from hybrid populations as separate collections into the VCS. If population monitoring showed a high level of fitness, the stored seed could be used for restoration in similar habitats. By recognising the unique contributions of hybrids, conservation efforts might enhance resilience and promote long-term ecological stability.

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