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Molecular and morphological analyses support recognition of *Prostanthera volucris* (Lamiaceae), a new species from the Central Tablelands of New South Wales

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ABSTRACT

Research into the systematics of *Prostanthera* recently revealed close evolutionary relationship among *P. phylicifolia sens. str.*, the critically endangered *P. gilesii*, and a population of uncertain identity from the Central Tablelands of New South Wales (NSW), Australia. Previous analyses were unable to establish whether genetic boundaries separated these taxa. This study assessed species boundaries among these three taxa by using a combination of single-nucleotide polymorphisms (SNPs) sampled at the population-scale and multivariate analysis of morphological characters. Ordination, model-based clustering, *F*-statistics, neighbour-network analysis, phylogenetic analysis, and ancestry coefficient estimates all provided support for discrete genetic differences among the three taxa. Morphological phenetic analysis recovered congruent morphological clusters and identified a suite of corresponding diagnostic characters. This congruence of molecular and morphological evidence supports the presence of three independently evolving lineages, two of which correspond with the previously described *P. gilesii* and *P. phylicifolia sens. str.* The third taxon, represented by a single population from the Central Tablelands of NSW, is here described as *P. volucris* R.P.O'Donnell. A detailed description, diagnostic line drawings and photographs are provided. We evaluate *P. volucris* as satisfying criteria to be considered *Critically Endangered*.

Keywords: critically endangered, DArTseq, genotyping-by-sequencing, Lamiaceae, population genomics, species delimitation, systematics, taxonomy.

Introduction

Prostanthera Labill. is the most speciose genus of endemic Australian Lamiaceae, encompassing over 105 accepted species (Conn 1984, 1988; Australian Plant Census 2021; Conn *et al.* 2021). Recent studies have shown the genus to be far more diverse than currently recognised, with several species complexes that require resolution (Wilson *et al.* 2012, 2019; Conn *et al.* 2016, 2021; O'Donnell *et al.* 2021*a*). Many species of *Prostanthera* are niche specialists and tend to grow on isolated, rocky outcrops (Conn 1984, 1988; Conn and Wilson 2015; Wilson and Conn 2015; Wilson *et al.* 2019). Like other rocky outcrop specialists, these species have highly restricted ranges and are particularly vulnerable to environmental threats (Fitzsimons and Michael 2017; Selwood and Zimmer 2020; Hopper *et al.* 2021; Silveira *et al.* 2021). Among the accepted species, 19 are listed as *Threatened* on the *Environment Protection and Biodiversity Conservation Act* List of Threatened Flora, representing almost one-fifth of the genus (see https://www.environment.gov.au/cgi-bin/sprat/public/publicthreatenedlist.pl?wanted=flora). One example is the *Critically Endangered P. gilesii* G.W.Althofer *ex* B.J.Conn & T.C.Wilson, which is geographically restricted in comparison with its close relative, *P. phylicifolia* F.Muell. *Prostanthera gilesii* is currently the subject of a targeted conservation and management project (NSW Department of Planning and Environment 2019; Scott and Auld 2020; H. C. Zimmer, J. J. Bruhl and R. L. Andrew, unpubl. data).

The species is currently known only from two small subpopulations within the Mount Canobolas State Conservation Area, south-west of Orange in the Central Tablelands of New South Wales (Conn and Wilson 2015).

Molecular phylogenies recovered a close relationship between *P. gilesii* and *P. phylicifolia s. str.*, whose distribution extends across the Victorian Alps and Snowy Mountains, Monaro, and Southern Tablelands of New South Wales (O'Donnell *et al.* 2021*a*) (Fig. 1). *Prostanthera phylicifolia* was originally described by von Mueller (1858) from material collected near Omeo in northern Victoria; however, the name has been misapplied since Bentham's (1870) circumscription of the species, which cited additional specimens from the New England region of New South Wales, and the Glass House Mountains, Queensland. Although specimens variously identified as *P. phylicifolia*

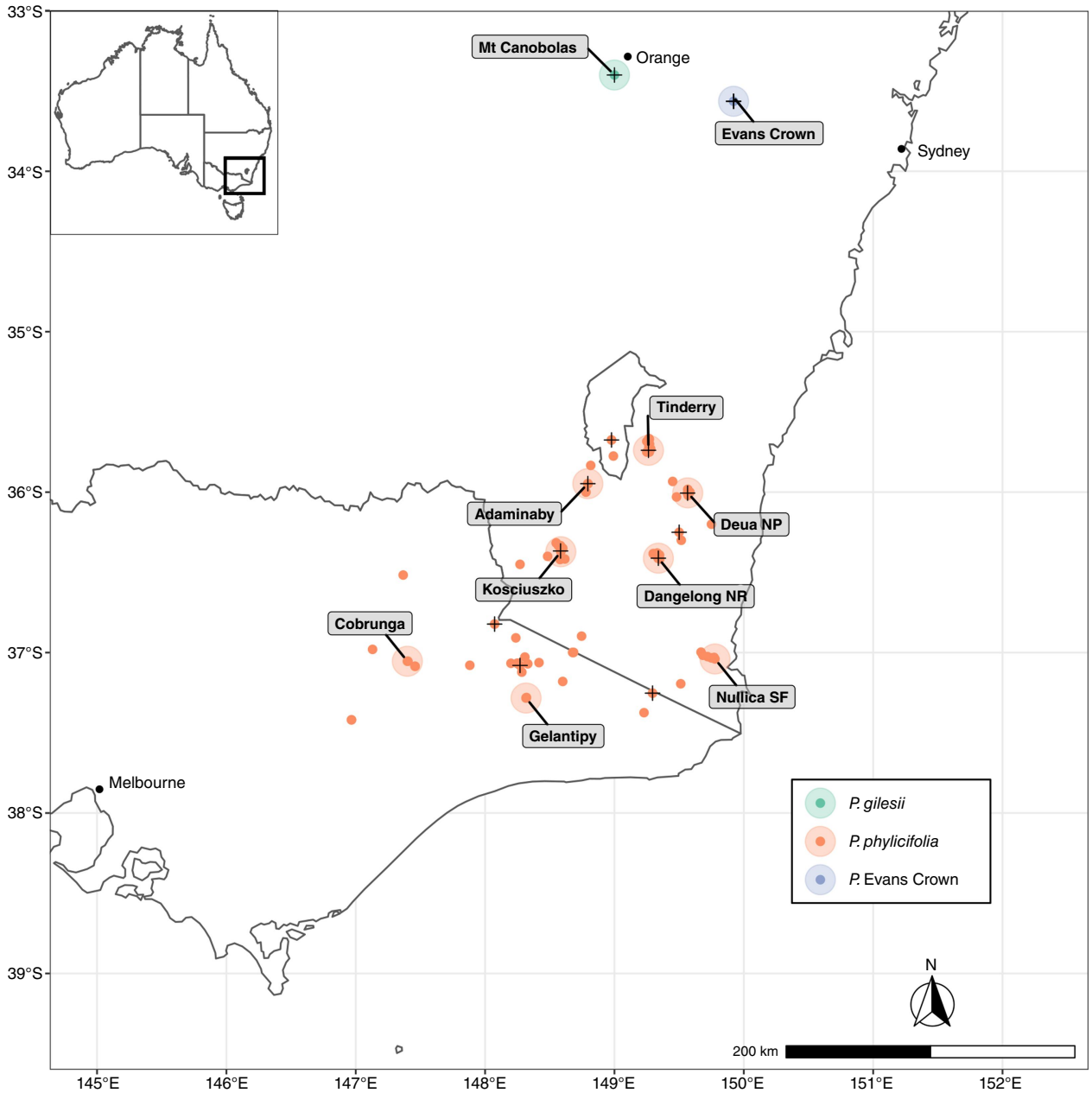

Fig. 1. Occurrence records of *Prostanthera gilesii*, *P. phylicifolia sens. str.* and the Evans Crown population obtained from Australia's Virtual Herbarium (2021) after removal of misidentified records and accessions of *P. phylicifolia s. lat.* as identified by O'Donnell *et al.* (2021). Populations sampled in this study for genomic analysis (Supplementary Table S1) are indicated with larger, transparent circles, and populations with associated herbarium vouchers that were measured for morphological phenetic analysis (Supplementary Table S2) are indicated with crosses.

may share glabrous, narrow ovate leaves, populations from Victoria and the Southern Tablelands of New South Wales differ substantially in their floral morphology from populations from the Northern Tablelands of New South Wales and southern Queensland. Southern populations have white corollas with purple and yellow punctate markings that are conspicuously zygomorphic, and anthers with a distinctly elongated connective tissue appendage between both pollen sacks. Conversely, northern populations exhibit entirely mauve corollas that are less strongly zygomorphic, and anthers with appendages that are reduced to absent. Wilson *et al.* (2017) demonstrated that these distinct floral types were correlated with different pollinator assemblages, and, moreover, indicative of phylogenetic relationship. Thus, species exhibiting differences in floral type were unlikely to be closely related. These results were supported by molecular phylogenies presented by O'Donnell *et al.* (2021*a*), which unequivocally demonstrated that *P. phylicifolia sens. str.* is restricted to Victoria and the Southern Tablelands of New South Wales, whereas populations from the Northern Tablelands of New South Wales and southern Queensland variously identified as *P. phylicifolia* represent an undescribed taxon more closely allied to *P. scutellarioides* (R.Br.) Briq. All further references to *P. phylicifolia* in this manuscript will refer to *P. phylicifolia sens. str.* as outlined above.

Another population of uncertain identification from Evans Crown Nature Reserve, located ~2.8 km south-east of Tarana, New South Wales, was also included in the study and recovered as sister to *P. gilesii*. Although superficially similar to *P. phylicifolia* and *P. gilesii*, the Evans Crown population is distinguishable in a number of ways that suggest it may also be a distinct species. The Evans Crown population differs substantially with respect to several reproductive and vegetative characters (O'Donnell *et al.* 2021*a*), and is found on granite outcrops (NSW National Parks and Wildlife Service 2009; R. P. O'Donnell, pers. obs., 2020), whereas its closest relative (*P. gilesii*) is found on basaltic substrates (Scott and Auld 2020). The differences exhibited by the Evans Crown population do not match diagnoses of *P. phylicifolia* or *P. gilesii* (von Mueller 1858; Conn and Wilson 2015). Tree topologies recovered by O'Donnell *et al.* (2021*a*) based on cpDNA or nDNA were not sufficiently resolved to confidently assess whether *P. phylicifolia* was reciprocally monophyletic or paraphyletic with respect to the *P. gilesii*-Evans Crown clade. Consequently, these data were considered insufficient to determine species-level boundaries.

The aim of this study was to elucidate the relationship among *P. gilesii, P. phylicifolia* and the Evans Crown population and to assess whether they each represent independently evolving lineages that warrant species-level recognition. Multiple individuals per population were sampled for each putative taxon to produce single-nucleotide polymorphism (SNP) data, which were then analysed using population genetic and phylogenetic methods. Morphometric phenetic analyses were then applied to objectively assess interspecific morphological variation and distinguish informative characters for identification.

Materials and methods

Integrative taxonomic approach

Integrative taxonomy aims to incorporate and synthesise multiple, independent lines of evidence to rigorously test and corroborate delimitation hypotheses, thereby increasing the probability that a set of independently evolving metapopulation lineages, i.e. species, *sensu* de Queiroz (2007), will be resolved (Dayrat 2005; Padial *et al.* 2010; Schlick-Steiner *et al.* 2010). To recover a stable taxonomic classification that agrees with evolutionary history, and to avoid the possibility of taxonomic over-inflation (Georges *et al.* 2018; Hundsdoerfer *et al.* 2019), an integrative taxonomic approach must be applied to questions of species delimitation in *Prostanthera*. Schlick-Steiner *et al.* (2010) provided a framework for an integrative taxonomic approach, suggesting that morphology be used as a primary line of evidence, followed by a genetic discipline. Previous studies of *Prostanthera* have incorporated phenetic analyses of morphological characters to rigorously assess phenotypic variation among putative taxa (Conn 1984; Conn *et al.* 2013, 2021; Wilson *et al.* 2017). Previous molecular studies of *Prostanthera* incorporating Sanger sequencing data have provided some species-level resolution (Wilson *et al.* 2012; Conn *et al.* 2013, 2016, 2021); however, O'Donnell *et al.* (2021*a*) and Wilson *et al.* (2012) recovered discordant topologies between nuclear and chloroplast datasets, suggesting that hybridisation, introgression, or incomplete lineage sorting may have occurred within *Prostanthera*. The use of high-throughput sequencing molecular approaches capable of detecting genome-wide admixture was recommended as a means for future studies of *Prostanthera* to mitigate the confounding effect of these evolutionary processes (O'Donnell *et al.* 2021*a*).

Genotyping-by-sequencing

DArTseq (Kilian *et al.* 2012) is a cost-competitive genotyping-by-sequencing (GBS) platform (Diversity Arrays Technology Pty Ltd (DArT), Canberra, ACT, Australia) that captures SNP data that have provided resolution and demographic inference at the population scale in taxonomically recalcitrant plant groups (Sansaloni *et al.* 2010; Steane *et al.* 2011; Joyce *et al.* 2021; Collins *et al.* 2022; Wilson *et al.* 2022). Genotyping-by-sequencing allows for a targeted fraction of an organism's genome to be sequenced in species with little pre-existing reference information (Narum *et al.* 2013; Soltis *et al.* 2013; Fernández-Mazuecos *et al.* 2017). In contrast to

microsatellite or amplified fragment-length polymorphism (AFLP) approaches, which rely only on a small number of neutral molecular markers representing a limited subset of the genome, GBS approaches greatly increase the number of putatively neutral markers assayed, thereby improving the precision of estimates of population structure, admixture, and demography (Narum *et al.* 2013). DNA extraction, library preparation and genotyping-by-sequencing using the DArTseq platform were conducted by DArT. The resulting reads were filtered, assembled *de novo* and scored by DArT internally, using their proprietary analysis pipeline following methods similar to those outlined by Kilian *et al.* (2012) and Cruz *et al.* (2013).

Sampling

Material was sourced from herbarium specimens or field collections of silica-dried or freeze-dried leaf material. *Prostanthera phylicifolia* was collected from 12 populations spanning its geographic distribution, *P. gilesii* was collected from both known subpopulations within the Mount Canobolas State Conservation Area (here referred to as Towac and Walls), and material was collected from the single known population located within the Evans Crown Nature Reserve (Fig. 1). Leaf material was also sampled from a herbarium voucher from Evans Crown (Rodd 11009, NSW 856887), collected from coordinates that do not match the immediate vicinity of the known population; however, the DNA yield for this specimen was too low for sequencing. The compact and tangled habit of *P. gilesii* and the Evans Crown taxon complicated an accurate estimation of the number of individuals. Samples were collected from plants at least 6 m apart to reduce repeated sampling of the same genet, but where separate individuals were clear, each was sampled (e.g. the Walls population consists of two individuals within a shared 3-m-diameter space). Herbarium vouchers were lodged at the National Herbarium of New South Wales (NSW) and the N.C.W. Beadle Herbarium (NE). Flowering material was collected and preserved in 70% ethanol for morphological examination.

To recover SNP data for population genetic analyses, 125 samples were sequenced and co-analysed by DArT. Of the 125 samples, 30 samples from the Evans Crown population, 7 samples of *P. phylicifolia* and 1 sample of *P. gilesii* (Walls) were newly sequenced for this study. New samples were co-analysed with samples of *P. gilesii* (62), *P. phylicifolia* (13), and the Evans Crown population (7) that had been previously sequenced as part of targeted conservation and management projects underway for *P. gilesii* (NSW Department of Planning and Environment 2019; Zimmer *et al.*, unpubl. data). Three samples from the same preliminary *P. gilesii* sequencing round were sequenced again as technical duplicates (Supplementary Table S1). One sample of *P. phylicifolia* that had previously been sampled as part of a targeted conservation and management project for *P. densa* A.A.Ham. and *P. marifolia* R.Br. was included in this co-analysis (Yap *et al.* 2020).

To provide outgroup representatives for phylogenetic analysis, an additional SNP dataset was co-analysed by DArT where selected samples of *P. gilesii* (4), *P. phylicifolia* (14) and the Evans Crown population (3) were co-analysed with samples of *P. densa* (2), *P. marifolia* (3), *P. granitica* Maiden & Betche (2) and *P. scutellarioides* (R.Br.) Briq. (2) that had previously been sampled as part of a targeted conservation and management project for *P. densa* and *P. marifolia* (Yap *et al.* 2020). These species were chosen as outgroup representatives because they were previously recovered within clades that were sister to the clade containing *P. gilesii*, *P. phylicifolia* and the Evans Crown population (O'Donnell *et al.* 2021*a*). In total, 30 samples were included for this analysis.

For morphometric phenetic analysis, 20 herbarium voucher specimens were measured and scored (Fig. 1, Supplementary Table S2). For both *P. gilesii* and the Evans Crown population, five voucher specimens were scored. For *P. phylicifolia*, 10 specimens were scored to cover the species' broader distribution. Each herbarium voucher specimen was considered as an individual plant and OTU when scoring. Voucher specimens were selected to incorporate the extent of variability within a taxon, and on the basis that they could provide three replicates for each character being scored.

Fieldwork was conducted under New South Wales Department of Planning and Environment Scientific Licence SL100305.

Molecular analysis of SNP data

The SNP dataset delivered by DArT was processed with the package *dartR* (ver. 2.0.4, see https://cran.r-project.org/package=dartR; Gruber *et al.* 2018; Mijangos *et al.* 2022) in the statistical package *R* (ver. 4.2.0, R Foundation for Statistical Computing: Vienna, Austria, see https://www.r-project.org/). Processing followed methods outlined by Gruber *et al.* (2018). First, the 'gl.filter.secondaries' function was used to remove secondary SNPs (i.e. sequenced fragments with more than one SNP). This function keeps one SNP from each fragment identified as having multiple SNPs. The SNP dataset was then filtered on the basis of read depth using the 'gl.filter.rdepth' function with the default settings. Sites were then filtered to require 98% reproducibility (as estimated by the DArT proprietary pipeline) by using 'gl.filter.reproducibility'. The remaining loci were filtered on the basis of a minimum call rate of 95% by using gl.filter.callrate. Individuals were then filtered on the basis of individual call rate with the same function, requiring a minimum call rate of 80%. The resultant dataset comprised 10 486 loci scored for 120 individuals, i.e. 5 individuals were removed by filtering (Table S1). Filtering methods were the same for the secondary dataset used for phylogenetic analysis, with the exception of filters for loci and

individual call rates. The minimum locus call rate was lowered to 80% to keep as many loci as possible and the minimum individual call rate was lowered to 60% to ensure that outgroup representatives were not filtered out. A total of 2 individuals were removed by filtering and the resultant dataset for phylogenetic analysis comprised 2519 loci scored for 27 individuals (Table S1).

Exclusion of clones and close relatives helps avoid bias in population genetic parameters in species with mixed sexual and asexual reproduction (Montalvo *et al.* 1997). To identify putative clones and mitigate their confounding effect in calculations of population statistics, the function 'gl.propShared' was used to calculate a similarity matrix for individuals for each population on the basis of their proportion of shared alleles. Pairwise shared allele frequencies between accessions that had a technical replicate sequenced were used to determine a threshold by which identical (i.e. clonal) accessions could be identified. The most conservative frequency of 0.9910912, between JJB3602ee and JJB3602ee.1, was used as the final cut-off threshold. Pairwise shared allele frequencies above the cut-off threshold were then counted for each accession. Accessions that returned zero pairwise shared allele frequencies above the cut-off were retained as distinct genotypes. The remaining non-zero accessions were then sorted into groups (distinct count value categories) by identifying accessions with the same number of total pairwise frequencies above the cut-off threshold.

The number of distinct count value categories was subtracted from the number of individuals sampled for that population, and count categories with counts below this number were each considered to represent genotypes with several clones present. For each remaining count category, the individual with the lowest mean pairwise frequency was retained for further analyses. Using pairwise shared allele frequencies, 58 samples of *P. gilesii*, 2 samples of *P. phylicifolia* and 20 samples of the Evans Crown population were excluded (Table S1) from further analyses. A detailed walkthrough of this process and the associated *R* script is available at https://github.com/rpodonnell/ASB_PEC.

To assess the similarities among populations and individuals, ordination of the dataset using principal-component analysis (PCA) was conducted using the 'gl.pcoa' function in *dartR*. To perform a neighbour-net network (Bryant and Moulton 2004) analysis and visualise a distance-based network, a Euclidian distance matrix was calculated using the 'gl.dist.ind' function in *dartR* and exported using the 'splitstree' function in *RSplitsTree* (ver. 0.1.0, B. Bickel and T. Zakharko, see https://github.com/IVS-UZH/RSplitsTree). The resulting distance matrix file was then imported to *SplitsTree4* (ver. 4.19.0, see https://software-ab.cs.uni-tuebingen.de/download/splitstree4/welcome.html; Huson 1998; Huson and Bryant 2006) where a neighbour-net network was calculated using the ordinary least squares (OLS) variance method and a lambda fraction of 1.0.

To produce a concatenated SNP matrix for phylogenetic analysis, SNP data were exported using the 'gl2svdquartets' function in *dartR* by using 'Method 2', which outputs a single line per sample and codes heterozygous SNPs or ambiguities by using standard ambiguity codes. To examine phylogenetic relationships, trees were estimated from the concatenated SNP matrix under a coalescent model by using SVDquartets (Chifman and Kubatko 2014, 2015) as implemented in PAUP* (ver. 4.0a169; Swofford 2002). Quartets were sampled exhaustively by using the Quartet FM (QFM) quartet-assembly algorithm, with 1000 standard bootstrap replications conducted to estimate branch support. Bootstrap support values were considered strong if they provided support values of $\geq$95%, moderate from 80 to 94% and weak from 50 to 79%. The final bootstrap consensus tree was visualised using the *R* package *ggtree* (ver. 3.6.1, see https://doi.org/doi:10.18129/B9.bioc.ggtree; Yu *et al.* 2017; Xu *et al.* 2022). Tree files output by PAUP*, including the final bootstrap consensus tree and all bootstrap replicate trees, are included in the supplementary dataset (available at https://hdl.handle.net/1959.11/53809).

To assess levels of genetic diversity, observed heterozygosity (H_O) and expected heterozygosity (H_E) values were calculated using the 'gl.report.heterozygosity' function in *dartR*, which calculates SNP heterozygosity. Statistics were calculated at putative species level on the basis of clusters observed in PCA and neighbour-network analyses, by using the unbiased estimate of H_E to account for limited and variable sample sizes. Statistics were also calculated for populations with five or more samples. To assess levels of genetic divergence, pairwise F_{ST} values were calculated for putative species groups, and between populations with five or more samples using the 'stamppFst' function in the *R* package *StAMPP* (ver. 1.6.3, see https://cran.r-project.org/package=StAMPP; Pembleton *et al.* 2013), which estimates pairwise F_{ST} values according to Weir and Cockerham (1984). To estimate statistical support, 1000 bootstrap replicates and confidence intervals were calculated. Confidence intervals were estimated using the percentile method, whereby a percentage (default 95%) of bootstrapped F_{ST} values around the mean F_{ST} are selected, and the minimum and maximum values are recorded as upper and lower confidence intervals (Pembleton *et al.* 2013). Both subpopulations of *P. gilesii* were treated as a single population in these calculations. Other populations with five or more samples that were used in calculations included the Dangelong Nature Reserve and Adaminaby populations of *P. phylicifolia*, and the Evans Crown population. F_{ST} values of <0.05 were considered to indicate low genetic differentiation, 0.05–0.25 indicated moderate genetic differentiation, and values >0.25 indicated pronounced differentiation (Freeland *et al.* 2011).

To investigate admixture, individual ancestry coefficients were estimated using sparse non-negative matrix factorisation (sNMF) in the R package *LEA* (ver. 3.10.0, see https://bioconductor.org/packages/release/bioc/html/LEA.html; Frichot and François 2015). The package uses cross-entropy

values to infer the probable number of ancestral populations (K) in the data, and to assign individuals to genetic clusters. The optimal number of ancestral populations was selected on the basis of the post-stabilisation of the steepest decline in cross-entropy values (Frichot and François 2015; van der Merwe *et al.* 2021). The 'snmf' function was executed for values of K = 1–8, with 50 replicates for each value of K.

Phenetic analysis of morphological data

The character list for morphometric phenetic analysis (Supplementary Table S3) consisted of 29 morphological characters, comprising 12 vegetative and 17 reproductive characters. Characters were selected from previous morphological studies of *Prostanthera* (Conn 1984; Williams *et al.* 2006; Conn *et al.* 2013), with additional characters being added following preliminary examination of specimens (i.e. indumentum direction and density). The mean of replicates (3 per specimen) for quantitative characters (23 in total) was used in morphometric analysis.

Reproductive characters included only calyx, prophyll and podium characters and did not include androecial or gynoecial characters because an insufficient number of specimens exhibited useful reproductive material to score such characters consistently. Measurements of vegetative characters were taken from dry herbarium vouchers. Calyx and prophyll characters were measured from rehydrated specimens except for three specimens of the Evans Crown population (*Taseski 853*, *O'Donnell 30*, *O'Donnell 55*) and one specimen of *P. phylicifolia* (*O'Donnell 61*), where calyx and prophyll characters were scored from flowers preserved in 70% ethanol. The final matrix (Supplementary Table S4) contained 29 characters scored for 20 specimens, including 23 quantitative and 6 qualitative characters.

The morphological data matrix was analysed in PATN (ver. 4.0, see https://patn.org/; Belbin and Collins 2013) by using default settings. All characters were weighted equally in each analysis with the Gower association metric, because it is suited to datasets that contain a combination of quantitative, binary, and qualitative characters (Sokal 1986). The unweighted pair-group method using arithmetic means (UPGMA) was used for cluster analysis because it is considered to accurately represent real distances between individuals in taxonomic datasets (Chappill and Ladiges 1992; Copeland *et al.* 2007). Semi-strong hybrid multidimensional scaling (SSH-MDS) was used for ordination analyses because it has been shown to accurately reflect phenetic patterns (Minchin 1987) and has frequently been used in recent morphological phenetic studies (Plunkett *et al.* 2009; Bean 2014; de Salas and Schmidt-Lebuhn 2018), including studies of *Prostanthera* (Conn *et al.* 2013, 2021).

Results

Principal-component analysis of SNP data

PCA ordination of SNP data (Fig. 2) organised samples as the following three general clusters: (1) all samples of the Evans Crown population as a single cluster; (2) all samples of *P. gilesii*, subdivided into two subclusters corresponding to their respective subpopulation of origin; and (3) all sampled populations of *P. phylicifolia*, subdivided into subclusters corresponding to their respective population of origin. Of the first

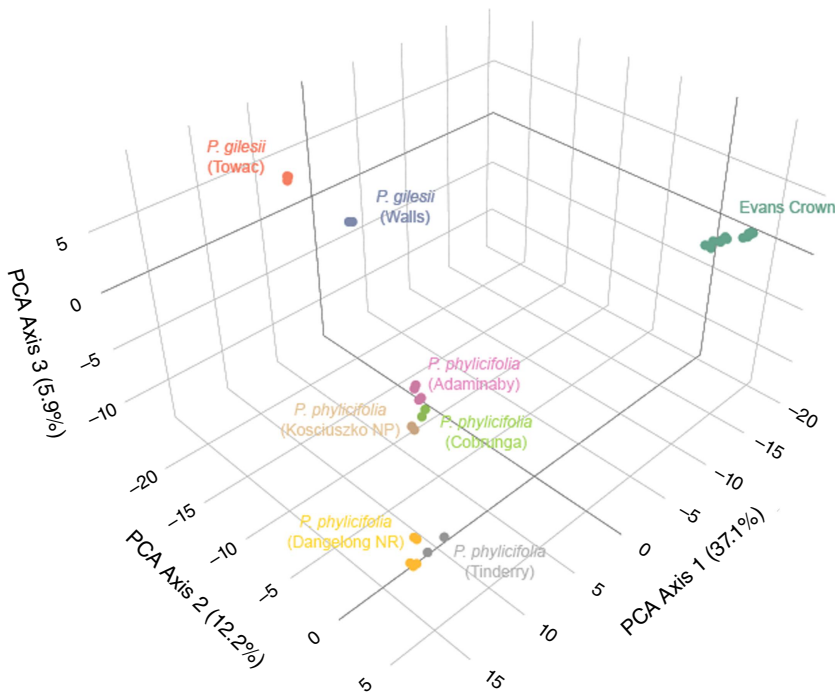

Fig. 2. Three-dimensional (3-D) plot of principal-component analysis (PCA) of DArTseq SNP data of samples remaining following the exclusion of clones, showing PCA1 *v.* PCA2 *v.* PCA3. NP, National Park; NR, Nature Reserve.

three axes, PCA Axis 1 explained 37.1% of variation, PCA Axis 2 explained 12.2% and PCA Axis 3 explained 5.9%.

Neighbour-net network analysis

The neighbour-net network graph (Fig. 3) showed three main branches exhibiting little to no reticulation between them. One branch included all samples of the Evans Crown population with some reticulation present among individuals. Another branch included all samples of *P. phylicifolia* and exhibited limited reticulation among individuals, but some reticulation between populations. Reticulation was observed within two main groups, namely, one consisting of individuals from Tinderry and Dangelong Nature Reserve, and another consisting of individuals from Adaminaby, Kosciuszko National Park and Cobrunga. The third branch included all individuals of *P. gilesii*, with both specimens from the Walls population forming a distinct subbranch. Little reticulation was present among individuals or between the two subbranches of *P. gilesii*.

Phylogenetic analysis

Exhaustive quartet sampling sampled 17 550 quartets in total, with a compatible quartet weight of 87.292% (15 318 quartets). The final bootstrap consensus tree (Fig. 4) recovered a strongly supported clade (BS = 100%) that contained all samples of *P. gilesii, P. phylicifolia* and the Evans Crown population, sister to *P. scutellarioides*. All samples of *P. phylicifolia* were recovered as a weakly supported clade (BS = 76%) sister to a strongly supported clade (BS = 100%) containing all samples of *P. gilesii* and the Evans Crown population. Within this clade, all samples of *P. gilesii* formed a strongly supported clade (BS = 100%) and all samples of the Evans Crown population formed a strongly supported clade (BS = 100%).

sNMF cluster analysis

On the basis of flattening of the cross-entropy curve (Supplementary Fig. S1), $K = 3$ was the most likely number of ancestral populations represented in the data. Estimates of ancestry coefficients for $K = 3$ recovered all samples of *P. phylicifolia* as one cluster, all samples of the Evans Crown population as a second cluster, and all samples of *P. gilesii* as a third cluster (Fig. 5). Each cluster exhibited little to no shared ancestry with other clusters. To compare alternative delimitation models, results for $K = 3$ were compared with results for $K = 2$ and $K = 4$. For $K = 2$; all samples of *P. phylicifolia* formed a cluster, and all samples of the Evans Crown population formed a cluster. Samples of *P. gilesii* were recovered as individuals with shared ancestry from both clusters. For $K = 4$, samples of *P. phylicifolia* were grouped into two clusters; the first comprised samples from Tinderry and Dangelong Nature Reserve, and the second comprised samples from Kosciuszko National Park,

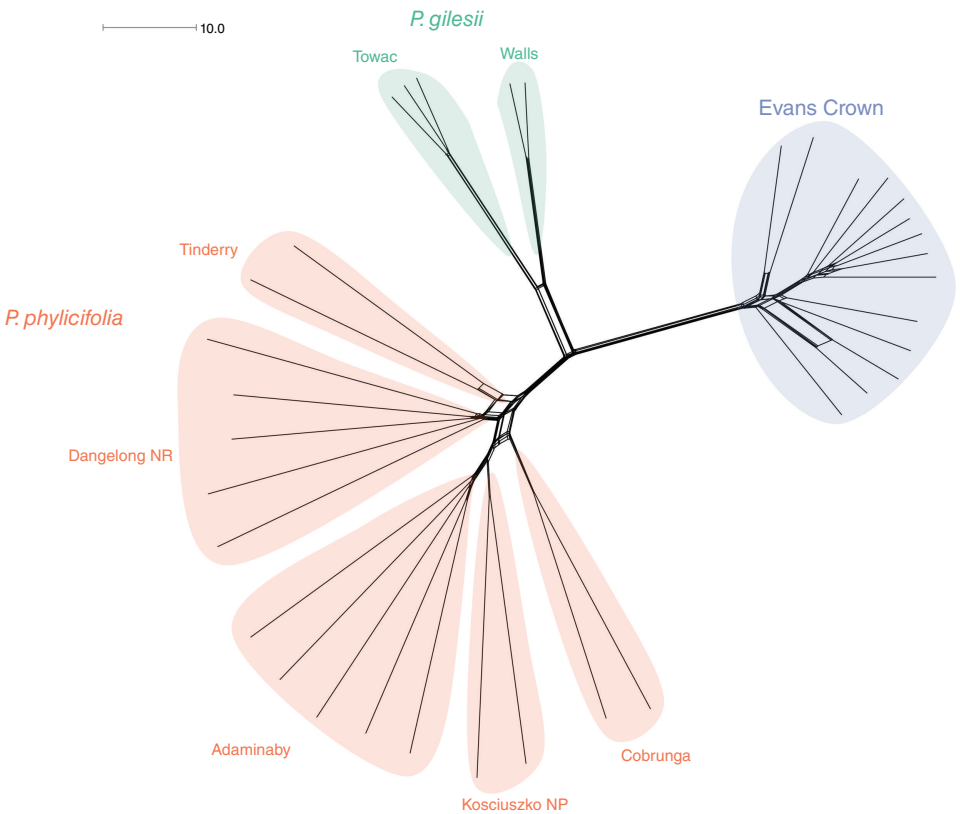

Fig. 3. Neighbour-network graph produced by SplitsTree5 of DArTseq SNP data of samples remaining following the exclusion of clones. Putative species groups are coloured, and populations are labelled. NP, National Park; NR, Nature Reserve.

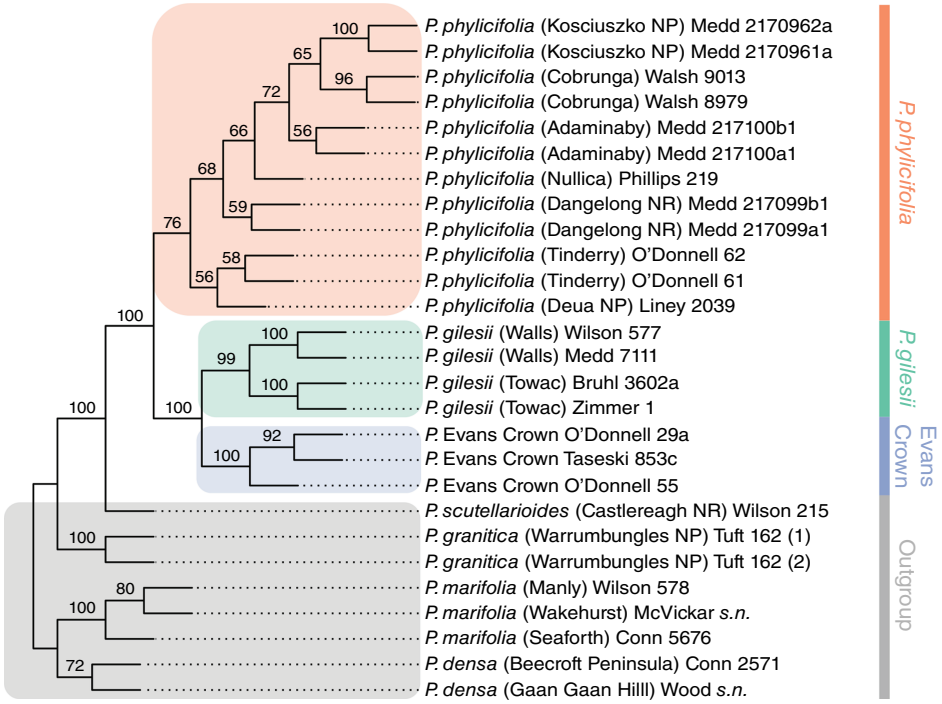

Fig. 4. Phylogeny generated by SVDquartets analysis of DArTseq SNP data for 27 samples of *Prostanthera*. Putative species groups are coloured, and populations are labelled. Labels are species/phrase names and population of origin, followed by primary collector and collection number.

Cobrunga and Adaminaby. Further clustering was seen within *P. phylicifolia* for *K* ranging from 5 to 7. Separation of both populations of *P. gilesii* was seen upward of $K = 6$.

Population genetic analysis

Following removal of putative clones, the highest population-scale F_{ST} value of 0.651 was observed between the Evans Crown population and the Adaminaby population of *P. phylicifolia* (Supplementary Fig. S2). The lowest F_{ST} value ($F_{ST} = 0.159$) was between the Dangelong and Adaminaby populations of *P. phylicifolia*, but all pairwise F_{ST} estimates were significantly greater than 0 ($P < 0.001$; Fig. S2). Observed and expected heterozygosity were highest in the Dangelong population of *P. phylicifolia* ($H_O = 0.172$; $H_E = 0.168$) and lowest in the Evans Crown population ($H_O = 0.054$; $H_E = 0.055$; Tables 1, 2). At the species level, the highest F_{ST} value of 0.630 was observed between the Evans Crown population and *P. gilesii*, whereas the lowest F_{ST} value of 0.306 was observed between *P. gilesii* and *P. phylicifolia* (Supplementary Fig. S3).

Morphometric analysis

Cluster analysis recovered three groups at a dissimilarity value of 0.2233 (Fig. 6*a*). The first group consisted of all specimens of the Evans Crown population, the second consisted of all specimens of *P. gilesii*, and the third consisted of all specimens of *P. phylicifolia*. The top five characters that contributed to the separation of these groups on the basis of Kruskal–Wallis values were lamina width, branch hair density, petiole hair direction, prophyll hair density and lamina length (Table 3).

Three discrete clusters were observed in SSH-MDS ordination plots; one cluster contained all specimens of the Evans Crown population; another contained all samples of *P. gilesii*; and the remaining cluster contained all samples of *P. phylicifolia* (Fig. 6*b*). The stress value of 0.0503 is interpreted as low, suggesting that the ordination is an accurate representation of the dataset in reduced dimensionality (Belbin and Collins 2013). Petiole hair direction and prophyll hair density, which were shown to be informative characters on the basis of Kruskal–Wallis values in the cluster analysis, were also recovered with R^2 values of >0.9 for the ordination (Supplementary Table S5).

Discussion

In this study, integration of genomic SNPs and morphometric analysis provided robust evidence in support of *P. gilesii* and the population from Evans Crown as distinct evolutionary lineages, and yielded insights into the population structure of the more widespread *P. phylicifolia*. O'Donnell *et al.* (2021*a*) first identified a close relationship among *P. gilesii, P. phylicifolia* and the Evans Crown population, but molecular data derived from Sanger sequencing were unable to satisfactorily resolve genetic boundaries among them. Here, analyses of genomic SNPs provide further support for the relationships recovered by O'Donnell *et al.* (2021*a*) (Fig. 4) and establish the presence of discrete genetic boundaries among the three taxa (Fig. 2, 3, 5, S2, S3). Our results demonstrated that they each form reciprocally monophyletic lineages that are strongly differentiated at many

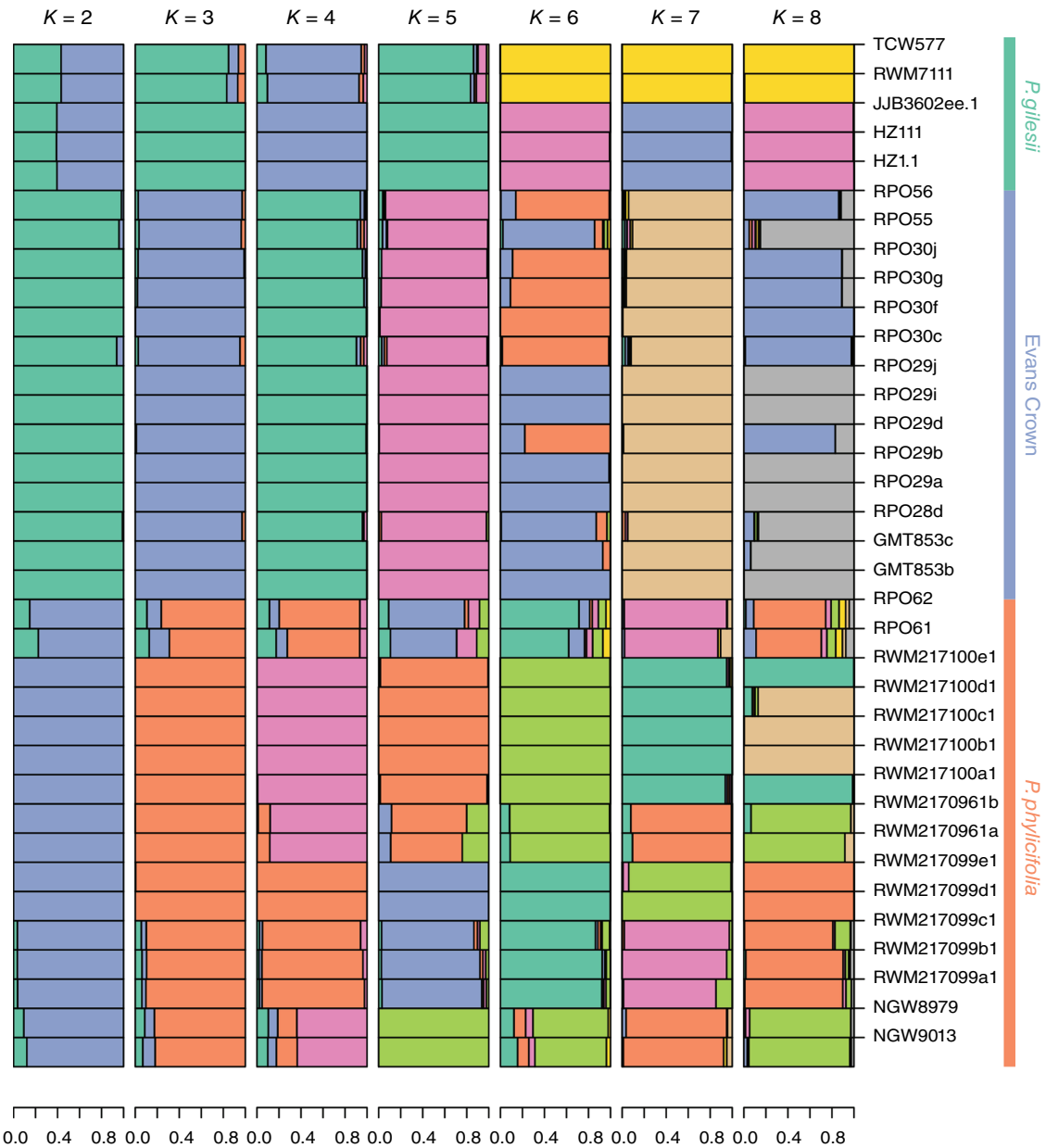

Fig. 5. Individual ancestry proportions from model-based clustering by using sNMF of all sampled individuals for values of $K = 2$–8. Putative species groups are labelled. Sample codes follow those outlined in Supplementary Table S1.

Table 1. Basic summary statistics for populations of *P. gilesii*, *P. phylicifolia* and the Evans Crown population where $n \geq 5$, following removal of putative clones.

Species	*n*	H_O	H_E
P. gilesii	5	0.109	0.091
P. phylicifolia (Adaminaby)	5	0.139	0.156
P. phylicifolia (Dangelong NR)	5	0.172	0.168
P. Evans Crown	14	0.054	0.055

nuclear loci and morphologically distinguishable, and are thus capable of being recognised as independently evolving lineages and discrete taxonomic entities, i.e. species.

Ordination analyses of SNP data (Fig. 2) recovered three core clusters congruent with the two named and one putative species. Both the neighbour-net network graph (Fig. 3) and sNMF ancestry coefficient estimate plots (Fig. 5) demonstrated little reticulation or recombination among the three groups, indicating that gene flow between these groups is highly restricted. Although phylogenies recovered

Table 2. Basic summary statistics for *P. gilesii, P. phylicifolia* and the Evans Crown population following removal of putative clones.

Species	*n*	H_O	H_E
P. gilesii	5	0.093	0.086
P. phylicifolia	16	0.116	0.168
P. Evans Crown	14	0.046	0.047

in this study (Fig. 4) and by O'Donnell *et al.* (2021*a*) both indicate a sister relationship between *P. gilesii* and the Evans Crown population, pairwise F_{ST} values between the two (Table 3, Fig. S3) suggest that they are more genetically differentiated from one another than either is from *P. phylicifolia*, despite the small geographic distance between *P. gilesii* and the Evans Crown population. Pronounced genetic differentiation among fragmented but geographically proximal populations and species has been similarly observed in other range-restricted granitic inselberg taxa (Hmeljevski *et al.* 2017; Bezemer *et al.* 2019; Robins *et al.* 2020). The observed heterozygosity values reported here (Tables 1, 2) suggest that accelerated genetic drift in historically small populations has likely contributed to the high F_{ST} values reported for *P. gilesii* and the Evans Crown population. The persistence of small populations is consistent with expectations for old, climatically buffered, infertile landscapes (OCBILs; Hopper *et al.* 2021). Nevertheless, the values reported here indicate substantial genetic divergence at the population and putative species level.

O'Donnell *et al.* (2021*a*) recovered two distinct clades of *P. phylicifolia sens. str.*, namely, a 'western' clade comprising populations occurring along the Victorian Alps and Snowy Mountains, and an 'eastern' clade comprising populations from Tinderry south to Dangelong. The phylogeny estimated in this study (Fig. 4) placed all samples of *P. phylicifolia* within a clade but did not recover the same eastern and western groupings. Neighbour-network analysis (Fig. 3) recovered clusters with a membership similar to the eastern and western clades recovered by O'Donnell *et al.* (2021*a*), but both branches exhibited moderate amounts of reticulation. Morphological analyses (Fig. 6) did not detect substantial variation among populations of *P. phylicifolia*; however, clusters recovered within this group did exhibit some geographic partitioning. For example, specimens from Mount Coopracambra, Tinderry and Deua National Park formed a cluster corresponding with the eastern clade, whereas samples from Wulgulmerang, Adaminaby and Kosciuszko National Park clustered together, corresponding with the western clade. Specimens from Dangelong Nature Reserve and Tuross Falls formed an intermediate cluster between these groups.

Within *Prostanthera*, similar east–west divergences across the same geographic regions were observed in morphological, phytochemical, and molecular analyses of the *P. lasianthos* Labill. complex (Conn *et al.* 2021). Morphological and phytochemical evidence supported the presence of distinct east–west phenotypes, albeit with marginal phylogenetic differentiation. Subsequently, a western lineage of *P. lasianthos* (congruent in distribution to the western clade of *P. phylicifolia*) occurring on the Southern Tablelands, predominantly within Kosciuszko National Park, was segregated as the new species *P. subalpina* B.J.Conn & K.M.Proft. Geographically partitioned genetic structure has been similarly observed in multiple plant and animal groups across the Australian Alps (Osborne *et al.* 2000; Mitrovski *et al.* 2007; Koumoundouros *et al.* 2009; Slatyer *et al.* 2014; Endo *et al.* 2015; Haines *et al.* 2017; Bell *et al.* 2018; Atkins *et al.* 2020; Sumner *et al.* 2021; Umbers *et al.* 2022) and the eastern Tallaganda–Monaro regions (Garrick *et al.* 2004, 2007, 2012; Bull *et al.* 2013; Carlson *et al.* 2016). The genetic signatures recovered by these studies are consistent with what would be expected following repeated glaciation events, and suggest that Pleistocene climatic oscillations and repeated glaciation of the Kosciuszko Massif and Australian Alps may have driven genetic diversity and lineage divergence within this region (Byrne *et al.* 2011; Endo *et al.* 2015; Bell *et al.* 2018).

Because geographically partitioned lineages have been observed in other taxonomic groups across the Australian Alps and Monaro regions, it is possible that the eastern and western groups of *P. phylicifolia* represent lineages that are either in the process of diverging, or, conversely, lineages that were once geographically isolated that have become reconnected. On the basis of the phylogenetic placement of the Nullica population of *P. phylicifolia* between putative eastern and western groups, it is possible that this population represents a point of historical contact between two diverging lineages; however, this too is difficult to assess given the limited sampling of this population in this study. Few studies have investigated the Australian Alps and eastern Tallaganda–Monaro region in tandem, or the corridors connecting them. Of studies that surveyed both regions, some east–west genetic partitioning has been observed, although, in most cases, the geographic scale of these studies was too broad to distinguish this pattern with confidence (Searle *et al.* 2000; Chapple *et al.* 2005; Symula *et al.* 2008; Worth *et al.* 2011). Bell *et al.* (2018) emphasised that patterns of population connectivity across the Australian alpine flora have not been thoroughly interrogated, and are subsequently poorly understood.

Although two separate clades of *P. phylicifolia s. str.* were recovered by O'Donnell *et al.* (2021*a*), the presence of gene flow and lack of substantial morphological differentiation demonstrated here suggest that these clades are likely to be representative of a single taxon not warranting further subdivision. All populations share a cohesive morphology, which is congruent with Mueller's protologue and type localities (von Mueller 1858). Our results therefore support the recognition of populations from the Victorian Alps and Snowy Mountains, Monaro, and Southern Tablelands of New South Wales as *P. phylicifolia sens. str.*, as originally

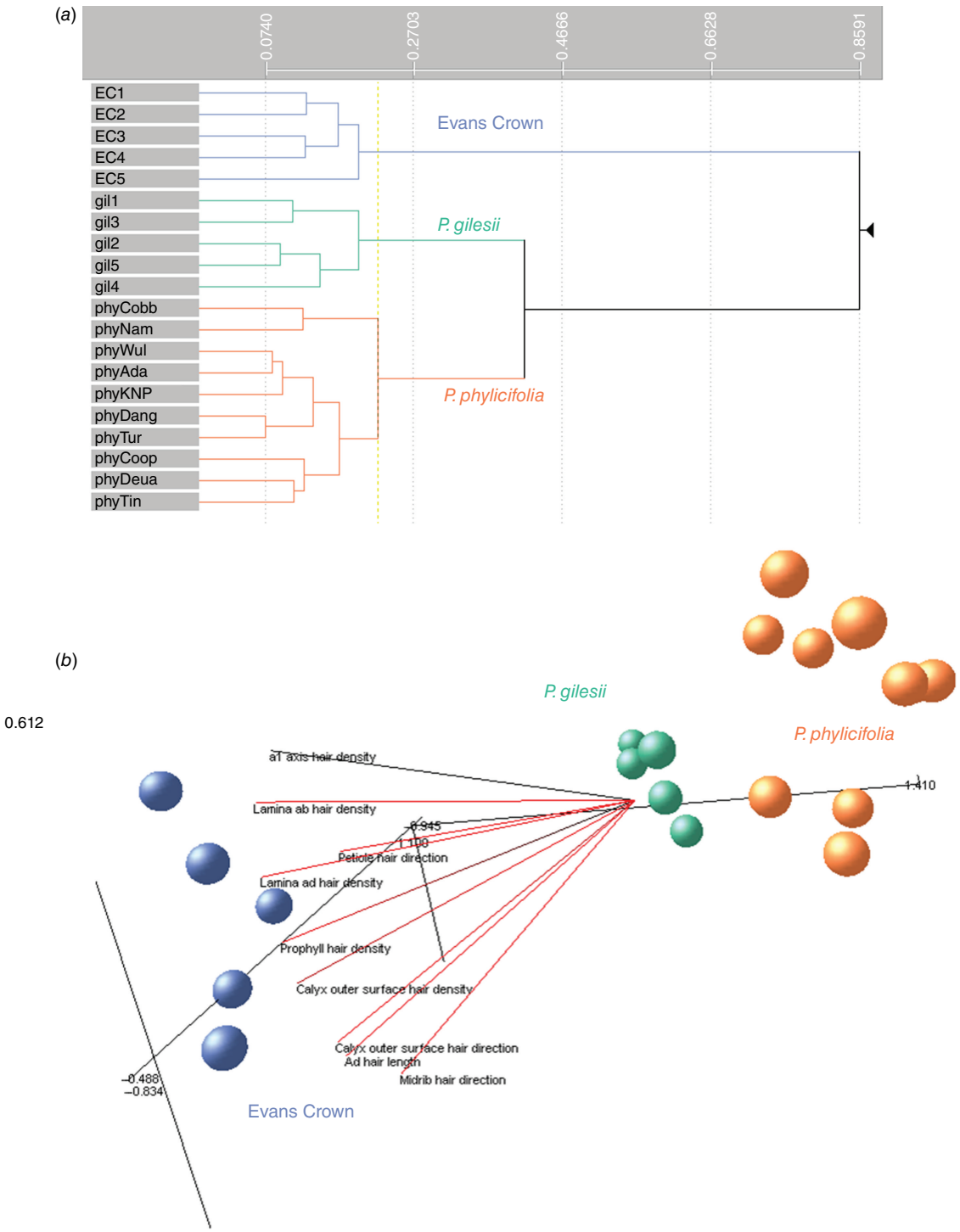

Fig. 6. (*a*, *b*) Output from morphometric analysis of 29 characters measured from 20 specimens of *Prostanthera*, showing three discrete groups. (*a*) Flexible unweighted pair-group method with arithmetic mean (UPGMA) phenogram, with yellow line indicating dissimilarity value; (*b*)semi-strong hybrid multidimensional scaling (SSH-MDS) ordination with characters, with PCC vectors with R^2 values of >0.9, with the size of each sphere representing its position in three-dimensional space (stress = 0.0503). See Supplementary Table S2 for OTU codes, Supplementary Table S3 for character list, and Supplementary Table S5 for PCC values.

Table 3. Top five characters contributing to distinction among groups in the phenetic cluster analysis in Fig. 6 based on Kruskal–Wallis (KW) values.

Character number	Character	Kruskal–Wallis
6	Lamina width	14.608
2	Branch hair density	14.143
4	Petiole hair direction	14.143
19	Prophyll hair density	14.143
5	Lamina length	14.071

published by von Mueller (1858). The remaining taxonomic quandary, then, is the question of how to treat *P. gilesii* and the Evans Crown population. O'Donnell *et al.* (2021*a*) proposed the following three possible options: (1) subsume *P. gilesii* and the Evans Crown population into an enlarged *P. phylicifolia*; (2) retain *P. phylicifolia* and *P. gilesii* as distinct species with the Evans Crown population synonymised with the latter; or (3) recognise all three taxa as distinct species. Although *P. phylicifolia, P. gilesii* and the Evans Crown population are closely related, gene flow among them is highly restricted, and the phenetic differences detected in this study suggest that they are each morphologically discrete entities. To treat all three as synonymous would fail to recognise the genetic and morphological diversity present. Phylogenetic analyses place *P. gilesii* and the Evans Crown population as sister taxa; however, molecular evidence presented in this study suggests that the Evans Crown population is more genetically distinct from *P. gilesii* than it is from *P. phylicifolia*. Treating *P. gilesii* and the Evans Crown population as synonymous would fail to represent the relationship between them accurately. On the basis of the congruence of evidence presented here, the most defensible position is to treat all three taxa as independently evolving metapopulation lineages (*sensu* de Queiroz 2007) that warrant species-level recognition. The Evans Crown population is demonstrably genetically and morphologically distinct from *P. gilesii* and *P. phylicifolia* and is, consequently, described here as *P. volucris* R.P.O'Donnell. *Prostanthera phylicifolia* is also lectotypified here to clarify the application of this name, and to restrict its usage to populations in southern New South Wales and Victoria, as per the findings of O'Donnell *et al.* (2021*a*).

Taxonomy

Prostanthera phylicifolia F.Muell. *Fragm.* 1(1): 19 (1858)

Type citation: 'In vertice rupestri montis McFarlane altitudine 4–5000', nec non in rupibus secus rivulos districtus Maneroo.' ['At the summit of rocky mountain McFarlane from altitudes of 4–5000', and also from cliffs or small rivulets in the district Maneroo']. *Type*: Australia: Victoria: Eastern Highlands: 'Mt M'Farlan', *s. dat.*, *F. Mueller s.n.* (lecto (here designated): K 000975463, left-hand side of sheet, right-hand sprig); isolecto: K 000975463, left-hand side of sheet, left-hand sprig; syn: MEL 43499 (right-hand side of sheet), MEL 43500).

Notes

There are several specimens of *P. phylicifolia* that are considered to be collections made by von Mueller (MEL 43499: left-hand side, MEL 43501, K 000975461); however, the handwriting on these respective accessions does not match that of Mueller's and is subsequently of uncertain authorship. Because it is unclear whether these accessions were indeed collected and inspected by Mueller, they are not considered to comprise part of the original material of this name. Specimen K 000975462 is a collection from a locality described in Mueller's handwriting as 'Mitta Mitta'. This locality is not mentioned in Mueller's protologue, and this specimen is therefore not part of the original material for this name.

Because O'Donnell *et al.* (2021*a*) demonstrated that *P. phylicifolia sens. str.* is restricted to southern New South Wales and Victoria, Mueller's locality description of 'Maneroo' must be considered to be an early spelling of Monaro (referring to the Monaro region), and not the rural locality of Maneroo located in Longreach, Queensland. Although specimens bearing the locality description 'Maneroo' (K000975460, K000975461) appear to be conspecific with the Mount McFarlane specimens, they have been excluded as syntypes because of the uncertain application of this locality name.

Specimen K000975461 bears a label of uncertain authorship reading 'var. velutina', and K000975462 is similarly labelled with 'v. velutina', albeit in this instance written definitively in Mueller's handwriting. Although Mueller's protologue describes individuals of *P. phylicifolia* as 'glabra v. velutina' (i.e. glabrous or velutinous; von Mueller 1858, p. 19), neither of these qualifiers are separated by Mueller as named varieties. Therefore, the name *P. phylicifolia* var. *velutina* appears to be a manuscript name of no nomenclatural standing that has never been published. O'Donnell *et al.* (2021*a*) found little genetic differentiation among individuals of *P. phylicifolia* that were predominantly glabrous and individuals that were densely hairy. Because most specimens of *P. phylicifolia* examined in this study were predominantly glabrous and some of the glabrous syntypes were well endowed with flowers and seemingly fruiting calyces, a representatively glabrous specimen is designated as the lectotype.

Prostanthera volucris R.P.O'Donnell, **sp. nov.** (Fig. 7, 8)

Type: Australia: New South Wales: Central Tablelands: Evans Crown Nature Reserve, ~2.8 km SE of Tarana township, 28 Oct. 2018, *G.M. Taseski 853*, (holo: NSW 1055966

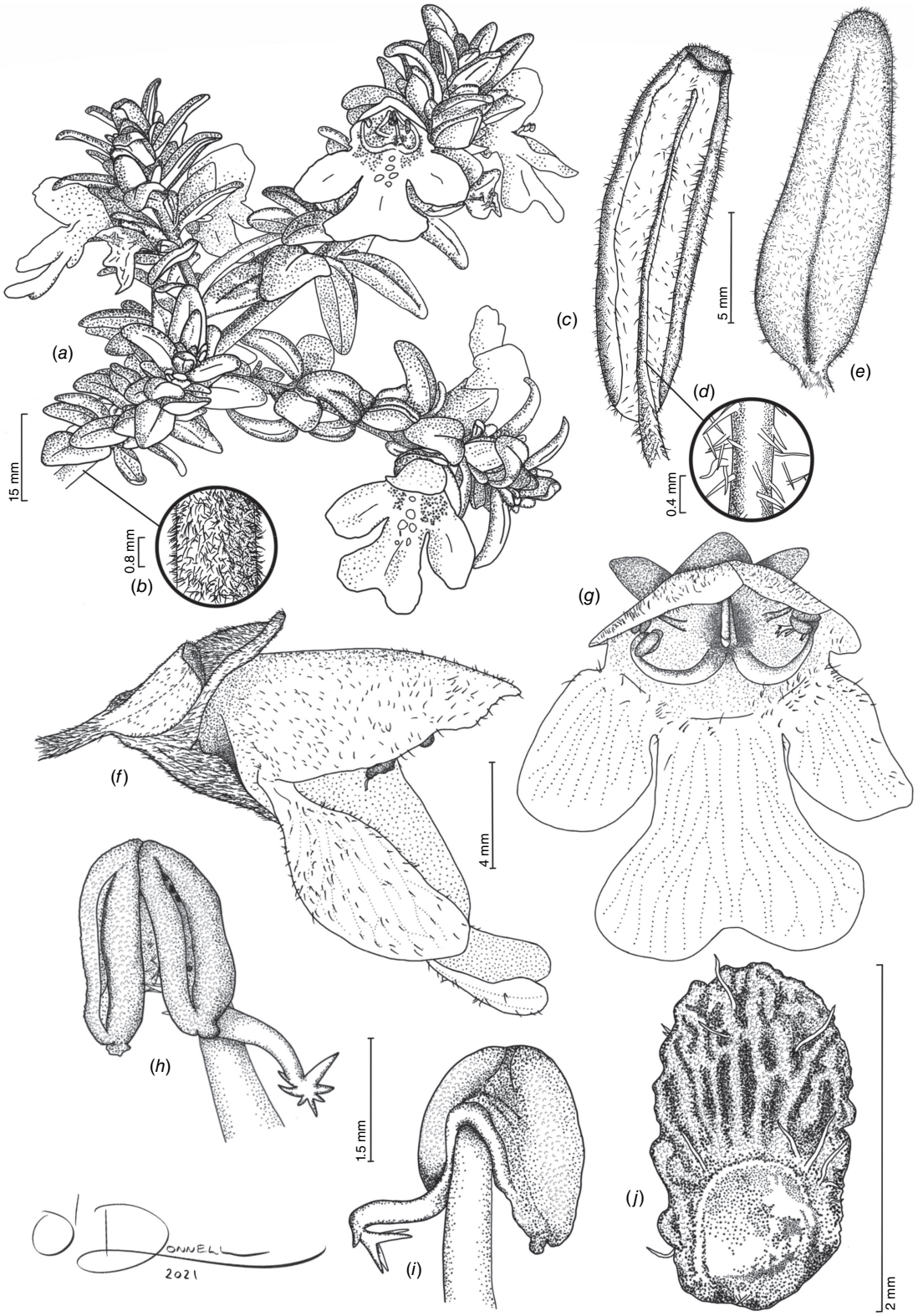

Fig. 7. (*Caption on next page*)

Fig. 7. Illustration of *Prostanthera volucris*. (*a*) Habit; (*b*) detail of branch surface, showing retrorse trichomes; (*c*) leaf surface, abaxial view; (*d*) detail of abaxial leaf lamina surface, showing midrib and indumentum; (e) leaf lamina surface, adaxial view; (*f*) flower, lateral view, showing calyx, prophyll, corolla, anthers; (*g*) flower, ventral view, showing corolla inner surface of lobes and tube, stamens, and style; (*h*) stamen, showing ventral view of anther locules, connective appendage and distal portion of staminal filament; (*i*) stamen, showing dorsal view of anther, connective appendage and distal portion of staminal filament; (*j*) mericarp, ventral view, showing abscission scar. Illustration: R. P. O'Donnell.

Fig. 8. Photograph images of *Prostanthera volucris*. (*a*) Habitat and associated vegetation; (*b*) habit; (*c*) habit, close-up; (*d*) flower and bud. Images: R. P. O'Donnell.

(sheet) and NSW 1057497 (spirit; listed as an associated collection on NSW 1055966); iso: BRI, CANB, K, MEL, MO, NE 110628, P, UNSW). *Prostanthera* sp. Evans Crown (G.M.Taseski NSW 1055966) (O'Donnell *et al.* 2021*a*).

Diagnosis

Prostanthera volucris can be distinguished from the morphologically similar *P. gilesii* by its larger prophylls, 3.8–9 mm long, 0.8–4.5 mm wide (*v.* <4 mm long, <0.6 mm wide for *P. gilesii*), densely hairy branches, calyces and prophylls (up to 80 *v.* up to 40 trichomes mm^{-2} for *P. gilesii*), appressed to subappressed retrorse trichomes (*v.* antrorse for *P. gilesii*) and densely hairy abaxial and adaxial lamina surfaces (*v.* predominantly glabrous with occasional antrorse trichomes restricted to the abaxial surface midrib for *P. gilesii*). *Prostanthera volucris* can also be distinguished from *P. phylicifolia* and *P. gilesii* by its mericarps that are rugose and papillose, with occasional long pilose trichomes (*v.* reticulate, not distinctly papillose and glabrous for *P.*

phylicifolia; mature mericarps have never been observed for *P. gilesii*; Table 4).

Compact, erect shrub up to 80 cm high, with plants forming tight mats. *Branches* ± terete, occasionally quadrangular early in development, becoming terete with age, densely hairy (20–80 trichomes mm^{-2}); trichomes 0.1–0.4 mm long, appressed to subappressed, retrorse, straight to slightly curled, white; sessile glands indistinct or absent (obscured by indumentum). *Leaves* velutinous, appearing silver–light green, slightly paler on abaxial surface, occasionally becoming red after prolonged exposure to strong sunlight, not aromatic when touched or crushed; *petiole* 0.88–1.4 mm long, densely hairy (20–48 trichomes mm^{-2}), the trichomes 0.2–0.8 mm long, appressed to subappressed, retrorse, straight to slightly curled, white; *lamina* narrowly ovate to elliptic, 12–19 mm long, 3–5 mm wide, with length to width ratio 2.6–5.3 and length of maximum width from base to total lamina length ratio 0.1–0.4; abaxial surface moderately to densely hairy, particularly on midrib (16–40 trichomes mm^{-2}), the trichomes 0.2–0.4 mm long, spreading to erect (occasionally retrorse along midrib), straight to slightly curled, white; abaxial lamina glands indistinct; adaxial surface moderately to densely hairy (16–30 trichomes mm^{-2}), the trichomes 0.1–0.3 mm long, spreading to erect, straight to slightly curled, white; adaxial lamina glands indistinct; base obtuse (occasionally appearing attenuate because margin more involute towards base); margin recurved; apex obtuse; venation indistinct, the midrib slightly raised on the abaxial surface. *Inflorescence* a frondose botryoidal conflorescence of monadic uniflorescences with 4–8-flowers per conflorescence. *Podium* a_1 axis 0.9–2.8 mm long, densely hairy (30–60[–88] trichomes mm^{-2}), the trichomes 0.1–0.2 mm long, appressed to subappressed, retrorse, straight to slightly curled, white, with glands indistinct or absent (obscured by indumentum); anthopodium absent or indistinct. *Pherophylls* not seen. *Prophylls* ± persistent, inserted near base of calyx, opposite, narrow-ovate to narrowly elliptic, 3.8–9 mm long, 0.8–4.5 mm wide, the length to width ratio 1.8–5.6, the length of maximum width from base to total lamina length ratio 0.3–0.9, densely hairy (16–56 trichomes mm^{-2}), the trichomes 0.1–0.2 mm long, appressed to subappressed, retrorse, straight to slightly curled, white, with glands indistinct or absent (obscured by indumentum); base slightly attenuate; margin entire; apex obtuse; venation indistinct. *Calyx tube* 1.5–2.1 mm long, light green, occasionally darkening to dark mauve with sun exposure; *abaxial lobe* ovate to broadly ovate, 2–4 mm long, 2–4 mm wide at base, the apex rounded to retuse; *adaxial lobe* ovate to elliptic, 3–5.3 mm long, 2.1–4.2 mm wide at base, the length to width ratio 1.2–1.4, the apex ± rounded; the adaxial lobe length to abaxial lobe length ratio ~1.3; outer surface densely hairy (24–64 trichomes mm^{-2}), the trichomes 0.2–0.35 mm long, appressed to subappressed, retrorse, straight to slightly curled, white, with glands indistinct or absent (obscured by indumentum); inner surface of tube glabrous; inner surface of lobes moderately to densely hairy near margin and apex, the trichomes appressed to subappressed, spreading to occasionally antrorse, straight to slightly curled, white. *Corolla* 14–18 mm long, white, with purple to dark mauve speckled markings on the inner surface of the tube and pale orange to yellow markings on base of abaxial median lobe; *tube* 3–5 mm long; *abaxial median lobe* broadly spathulate, 7–9.5 mm long, 4–7 mm wide (below distal lobing), length to width ratio 1.4–1.8, the apex slightly irregular and

Table 4. Selected morphological attributes separating *Prostanthera volucris*, *P. gilesii* and *P. phylicifolia*.

Morphological character	*P. volucris*	*P. gilesii*	*P. phylicifolia*
Stem indumentum	Densely covered by appressed to spreading retrorse trichomes	Sparsely to moderately hairy with appressed antrorse trichomes	Glabrous to sparsely hairy with short appressed antrorse trichomes restricted to either nodes or decussate grooves
Lamina size	12–19 mm long, 3–5 mm wide	15–26 mm long, 6–10 mm wide	5–15 mm long, 1.5–4 mm wide
Lamina abaxial surface indumentum	Moderately to densely hairy across whole surface, midrib trichomes spreading to retrorse	Glabrous, occasionally with patches of long antrorse trichomes restricted to the midrib	Glabrous, occasionally with short appressed antrorse trichomes along the length of the midrib
Calyx	Densely covered in appressed retrorse trichomes, sessile glands indistinct or absent	Glabrous, moderately covered by sessile glands	Glabrous, occasionally with patches of short appressed to spreading antrorse trichomes, sparsely to moderately covered by sessile glands
Prophyll	Elliptic, densely covered in appressed to subappressed retrorse trichomes	Narrowly elliptic, glabrous except for a few antrorse trichomes restricted to the margin	Linear to terete, glabrous
Pedicel	Densely covered in appressed to spreading retrorse trichomes	Glabrous to sparsely hairy, trichomes antrorse	Glabrous
Mericarp	Rugose, papillose, with occasional long pilose trichomes	Mature mericarps not observed	Reticulate, glabrous

rounded, bilobed (sinus 2–2.3 mm long, 2.5–3 mm wide distally); *lateral lobes* oblong to slightly elliptic, 5.4–5.7 mm long, 3.1–3.8 mm wide, length to width ratio 1.5–1.8, the apex rounded, slightly irregular; *adaxial median lobe-pair* broad to depressed-ovate, 6–7 mm long, 9–9.5 mm wide, length to width ratio 0.6–0.8, the apex rounded, irregular, bilobed (sinus 0.6–0.8 mm long, 0.9–1.6 mm wide, the median margin of lobes occasionally overlapping slightly); outer surface sparsely glandular, moderately hairy, particularly on lobes (8–24 trichomes mm^{-2}), the trichomes 0.1–0.4 mm long, erect to spreading along tube before becoming appressed to subappressed and antrorse on lobes; inner surface ± glabrous, the lobes sparsely hairy to moderately hairy at the tube opening rim and sinuses between lobes, the trichomes 0.1–0.2 mm long, crinkled. *Stamens* inserted 2.8–4.1 mm above base of corolla; *filaments* 3.1–5.4 mm long, white, often with mauve tinge; *anthers* 1.4–1.8 mm long, minutely papillose with an acumen at the base of each lobe and trichomes between lobes, the trichomes 0.1–0.2 mm long, white; connective appendage 0.8–1.5 mm long, dark mauve maturing to yellowish-brown, with a few narrowly triangular trichomes that are 0.1–0.35 mm long and white. *Disc* 0.5–1 mm long. *Pistil* 7.5–9.5 mm long; *ovary* cylindrical-obovoid, 0.9–1 mm long, at base 0.9–1 mm diameter, the lobes 0.5–0.65 mm long; *style* 7.5–8 mm long; *stigma lobes* 0.3–0.4 mm long. *Fruiting calyx* not strongly accrescent, the abaxial lobe clasping to conceal developing mericarps, the adaxial lobe not strongly reflexed. *Mature mericarps* 1.8–2.1 mm long, 1–1.2 mm wide, rugose, minutely papillose, with a few spreading trichomes that are 0.2–0.5 mm long and white.

Distribution

Known from a single granitic tor in the Evans Crown Nature Reserve, south-east of Tarana, New South Wales, Australia (Fig. 1). This location is situated within the Central Tablelands Botanical Division and South Eastern Highlands IBRA Region (NSW National Parks and Wildlife Service 2009).

Habitat

Prostanthera volucris grows on exposed granite formations at 1000–1020 m altitude, along drainage crevices in shallow, skeletal humic soils, with *Cyphanthera albicans* and *Cheilanthes sieberi* nearby.

Etymology

From the Latin '*volucris*' ('winged' or 'winged creature'), in reference to the substantial and densely hairy prophylls, which, inserted at the base of the calyx, give the calyx the appearance of being winged.

Ecology and conservation

Although the first collector of *P. volucris* described it as 'locally abundant in rock crevices' (McKee 7043, NSW 237164), only one population is known. More intensive surveys of the Evans Crown Nature Reserve and other unexplored vegetation islands in the nearby area should be undertaken. Because *P. volucris* is known to grow only on exposed, granitic outcrops, future attempts to locate additional populations should prioritise these landscape features.

The pollinators of *P. volucris* are unknown; however, its floral characteristics correspond with a floral type visited primarily by bees, although not excluding other insects such as flies (Wilson *et al.* 2017). Studies of foraging ranges in Australian bees found a typical maximum foraging range of ~700 m (Smith *et al.* 2017), which suggests that pollen is unlikely to travel further than this distance. Because *P. volucris* grows in tight, tangled mats, it is difficult to determine the limits of individual plants. Pairwise shared allele frequencies indicate that some samples represent ramets from a clonal parent, whereas others represent separate individuals. Seedlings have been observed in recent surveys and mature individuals have been observed to set seed; however, it is unknown what proportion of seed set is viable. Because *P. volucris* is known to occur along drainage crevices, it is likely that seeds are being dispersed primarily by water runoff.

It is unknown whether *P. volucris* is subject to herbivory; however, as other species of *Prostanthera* have been observed to be grazed by herbivores, similar threats may apply in this instance (Jusaitis 2018). Sheep and goats have been known to access the reserve from neighbouring properties and there are currently no feral animal control programs implemented for the reserve (NSW National Parks and Wildlife Service 2009). The reserve is a popular attraction for hikers and rock climbers, and because the species occurs on a geologically striking outcrop, human activity is a likely threat to this population. On revisiting the site following the severe drought conditions of 2019–2020, the population appeared to be severely affected by heat and drought stress and had declined substantially in condition (G. M. Taseski, pers. obs., 2020). Projected prolonged drought conditions and heightened temperatures may adversely affect this population, particularly on account of its highly exposed habitat.

Given the horticultural potential of *P. volucris*, this species could be a target of increased collecting pressure. Attempts are underway to develop an *ex situ* collection with botanic gardens and native plant wholesalers. The known distribution of this species is restricted such that the area of occupancy and extent of occurrence are not greater than 4 km^2. Because this species is known from only one highly restricted population of <250 mature individuals where decline in response to drought stress has been observed, we suggest that this species satisfies the criteria to be considered *Critically Endangered* under the *New South Wales Biodiversity Conservation Act* 2016 (see https://www.legislation.nsw.gov.au/view/html/inforce/current/

act-2016-063#statusinformation), the *Environment Protection and Biodiversity Conservation Act* 1999 and the *IUCN Red List* criteria thresholds (International Union for Conservation of Nature and Natural Resources 2022).

Notes

Although quantifiable mericarp characters were not included for morphological analyses, differences in mericarp morphology were observed between *P. volucris* and *P. phylicifolia*. The mericarp is rugose and distinctly papillate in *P. volucris*, whereas it is reticulate and not distinctly papillose in *P. phylicifolia*. Scant attention has been paid to mericarp surface ornamentation and sculpting in recent descriptions of *Prostanthera*, with the exception of Williams *et al*. (2006) in their treatment of the *P. spinosa* complex and Guerin's (2005) study of mericarp morphology in the Westringieae. The findings outlined here and by Guerin (2005) and Williams *et al*. (2006) highlight that mericarp morphology may be taxonomically informative in *Prostanthera*. Further examination of mericarp morphology across the genus is warranted and may provide additional characters for distinguishing among closely related species in future diagnoses.

Other specimens examined

AUSTRALIA: NEW SOUTH WALES: CENTRAL TABLELANDS: *South Eastern Highlands*: Evans Crown Nature Reserve: *H.S. McKee 7043*, 10 Jan. 1960 (NSW237164); *A.N. Rodd 11009*, 9 Mar. 2002 (NSW856887); *R.P. O'Donnell & G.M. Taseski 28*, 7 Apr. 2020 (NSW1100357); *R.P. O'Donnell & G.M. Taseski 29*, 7 Apr. 2020 (NSW1100369); *R.P. O'Donnell & G.M. Taseski 30*, 7 Apr. 2020 (NSW1100379); *R.P. O'Donnell & T.C. Wilson 55*, 4 Oct. 2020 (NSW1100402); *R.P. O'Donnell & T.C. Wilson 56*, 4 Oct. 2020 (NSW1100403).

Supplementary material

Supplementary material is available online.

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Data availability. All *R* code used in this study is available at https://github.com/rpodonnell/ASB_PEC. SNP data and metadata are available at the Research UNE data repository at https://hdl.handle.net/1959.11/53809. This paper was first made available as a preprint (O'Donnell *et al.* 2021*b*).

Conflicts of interest. Jeremy J. Bruhl is an Associate Editor of *Australian Systematic Botany* but did not at any stage have editor-level access to this manuscript while in peer review, as is the standard practice when handling manuscripts submitted by an editor to this journal. *Australian Systematic Botany* encourages its editors to publish in the journal and they are kept totally separate from the decision-making processes for their manuscripts. The authors have no further conflicts of interest to declare.

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